BIOREMEDIATION OF TCE BY MULCH BIOBARRIERS: IMPACTS OF THE PRESENCE OF CO-CONTAMINANTS

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ABSTRACT

Trichloroethene (TCE), a recognized human carcinogen, is a wide-spread groundwater contaminant and is commonly used as an industrial chlorinated solvent. It has been found in almost 34% of the nation's drinking water supplies. Except for ethene, all other daughter products from reductive dechlorination of TCE are toxic to human health. Starting from the past few decades, people have been exploring effective and efficient removal technologies of TCE from water resources. Among them, biological PRBs (Permeable Reactive Barriers), also called biobarriers, are a promising and cost-effective bioremediation treatment that passively capture contaminants plume and then remove, transform, or destruct contaminants into nontoxic chemicals before they leave the target site.

Despite the promise of applying biobarriers for bioremediation of chlorinated ethenes, no pilot- or field-scale research have demonstrated a complete dechlorination of TCE to benign ethene with a 100% conversion efficiency. Besides, several studies have demonstrated that the presence of nitrate and/or sulfate could preclude the reductive dechlorination of TCE under anaerobic conditions. Therefore, when remediating groundwater pollution with biobarriers, the presence of common nutrients including nitrate and sulfate and of other harmful industrial waste must be considered as complicating factors.

In this research, column- and batch-scale tests were conducted to observe and measure the dechlorinating performance of KB-1TM mixed culture in simulated lab-scale biobarriers and in batch tests, with the presence of diverse co-contaminants: nitrate, sulfate and RDX. Considering the favorable market price and higher adsorption capacity for chlorinated ethenes, pine bark mulch was chosen to be filled into PRBs in this research to act as substrate and serve as the electron donor to favor the growth of microbes of interest. Butyric acid was injected into both columns and batch

microcosms to enhance the reductive dechlorination by driving oxygen levels down and serving as extra electron donors. Gas chromatography was applied to compare methane production and quantify chlorinated ethenes inside columns and microcosms. PCR amplification and further DNA sequencing analysis were conducted to explore differences of microbial distributions along and between columns receiving varying electron acceptors.

Column-scale experiments turned out that sulfate with a concentration of 0.25 mM had no obvious inhibitory impact on the dechlorination ability of mulch biobarrier when receiving 1 mg/L TCE. Batch-scale tests revealed that the presence of 1 mM nitrate would decline the dechlorination rate of chlorinated ethenes, while the presence of 1 mg/L RDX seemed to make no difference. Batch-scale tests also indicated that the presence of chlorinated ethenes would inhibit the denitrification process. Results from DNA sequencing showed that significantly higher amounts of dechlorinators existed in columns without the presence of nitrate and sulfate, indicating that these two alternative electron acceptors are driving the differences of microbial diversity between control and experimental columns.

BIOGRAPHICAL SKETCH

Yating came to the United States to pursue her master degree in Environmental Engineering in September 2017. Before coming to Cornell University, she has obtained two Bachelor of Science degrees in Environmental Science from Queen's University, Canada and Tongji University, China.

Going through college, she has been mastering knowledge in the field of environment, and has applied the theoretical knowledge to her research projects and internship experiences in the investment bank Morgan Stanley and engineering consulting firms including AECOM.

Her life in Cornell University and in the United States is incredible and memorable. She benefits tremendously from her past experiences and she always expects to explore more to make a real difference to our environment.

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Chapter I Introduction and Background

1.1 Introduction

Chlorinated ethenes, including trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC), are groups of toxicants and among the most common organic groundwater contaminants. These chlorinated compounds are not thought to naturally occur in the environment, however, have significantly attracted public concern in the past few decades due to their negative health effects, wide use, uncontrolled disposal and loose management (Westrick et al., 2010).

TCE, chlorinated ethene with three chlorine atoms on the alkene, is a halocarbon commonly used as an industrial solvent. It is clear, non-flammable liquid with a sweet smell and a burning taste (U.S. Department of Health and Human Services, 2005). During the reductive dechlorination, a degradation process of chlorinated organic compounds with release of inorganic chloride ions that replaced by electrons coupled to hydrogen atoms by reductive dehalogenases, sequential dechlorination occurs from TCE to DCE to VC to non-toxic ethene (Dugat-Bony et al., 2012). Cis-1,2-dichloroethylene (cis-DCE) is the most commonly formed isomer of DCE in this reductive process.

More importantly, except for ethene, TCE and its daughter product cis-DCE and VC which are transformed by natural reduction, are proved to severely threaten human health as well as other living creatures, including risks of cancer. TCE, cis-DCE, VC, are all highlighted in the United States Environmental Protection Agency (USEPA) and Agency for Toxic Substances and Disease Registry Substance Priority List (ATSDR), with TCE and VC listed on the top 40 (ATSDR, 2011). Both TCE and VC are confirmed human carcinogens (U.S. Department of Health and Human Services, 2005) and VC poses an even more significant threat to human and nature. Therefore, not

only effective but also complete dechlorination of these dangerous chlorinated ethenes in groundwater has been critical to the public in the last several decades.

Treatment of TCE in groundwater has practically adopted both ex situ and in situ remediation technologies, including oxidative, reductive, biotic and abiotic methods. In recent years, when remediating TCE-contaminated sites, ex situ methods such as pumping and excavation are increasingly being replaced by less invasive in situ methods such as bioaugmentation and biostimulation with considerable successes (Dugat-Bony et al., 2012). Because of the highly-oxidized status of TCE, reductive dechlorination is more favorable than oxidative treatment. Reductive dechlorination has been successfully applied to many aquifers to improve the degradation of chlorinated ethene (Scheutz et al., 2008). When involving the addition of selective electron donors, the biostimulation technology can improve biodegradation if there exist microorganisms that are capable of complete dechlorination (Muller et al., 2004).

While microbes from many genera have been proved their capacity of catalyzing the dechlorination of TCE to DCE, Dehalococcoides (Dhc) is the only genus that is known to be able to completely reduce TCE to DCE and VC and then to the harmless end-product ethene (Futagami et al., 2008; Hendrickson, 2002). Thus, in order to avoid the stalling of reductive dechlorination at an intermediate stage where seriously resulting in an accumulation of cis-DCE and VC, daughter products known to be more toxic than TCE, cultures containing Dhc strains are needed and often added at TCE-contaminated sites to bioaugment natural micro-populations, i.e. bioaugmentation strategies. Among in situ bioremediation technologies, mulch biobarrier is one of the most promising bioremediation options for dilute concentrations of chlorinated contaminants in groundwater plumes occurring near the surface.

Moreover, our natural environment is not composed of a single element and is ever-changing: there would be other elements existing in the groundwater flowing through the TCE plume, including some common nutrients such as nitrate and sulfate, or even including explosive residuals. The presence of these co-contaminants might accelerate, inhibit or pose no noticeable impact on the process of reductive dechlorination. Therefore, further studies are needed to explore and understand the potential impacts of the presence of multiple co-contaminants to achieve a prospective bioremediation strategy of TCE by mulch biobarriers.

1.2 In Situ Groundwater Remediation Technologies

In situ remediation technologies indicate that the locations of the remediation occur on site. In situ treatments take place in contamination source zones or around the groundwater contaminant plumes, without the translocation of the polluted materials. Common in-situ remediation strategies contain physical removal, chemical destruction, and biological activities.

1.2.1 In Situ Remediation: Physical and Chemical Treatment

Air sparging is useful to remove volatile organic contaminants (Ellis et al., 2000), a method requiring the injection of a gas (usually air) into the saturated soil zone below the lowest level where there is no known contamination, after which the air will thoroughly contact with contaminants and strip these toxicants away or stay and act as an assistant. Then, air with contaminants will often be collected at the vadose zone by a soil vapor extraction system and ultimately treated on-site (Reddy, 2001). The overall cost of air sparging could be lower than pumping, however, low permeability of stratified soil might cause severe technical difficulties. The risk that contaminants spreading into clean regions also exists.

Taking advantaging of some contaminants' high Henry's Law constants, **air stripping** combined with carbon adsorption removes contaminants by moving them from their dissolved phase into air phase due to their high volatility, after which these contaminants released into the atmosphere or adsorbed by activated carbon (Rusell, et al., 1992). However, the cost to replace activated carbon sorbent could be really high.

Soil flushing is to inject the functional solution into groundwater by specific injection wells. The solution flows through the contaminated area and then adsorbs, solubilizes or flushes the toxicants from aquifers. Eventually, the solution carried with contaminants will be pumped out and treated above the surface (Roote, 1997).

Chemical oxidation can be applied either above ground or underground. Iron(III)-assisted permanganate and persulfate are popularly used to degrade TCE (Liang et al., 2004). Chemical oxidations usually occur fast, which is the strength of this remediation method. However, in order to evenly distribute the chemicals needed, multiple injections are often required, generating a high cost later (Huling & Pivetz, 2006).

1.2.2 In Situ Remediation: Permeable Reactive Barriers (PRBs)

PRBs, a relatively new developing and cost-effective remediation technology for in situ groundwater treatment, are porous barriers that passively capture contaminants plume and then remove, transform, or destruct contaminants into nontoxic chemicals (Gillham et al., 2010). PRBs are constituted by a wall of materials, with either soil or non-soil solid subjects, such as mulch or compost (Reddy, 2008). A simplified figure of a typical PRB system is shown below in Figure 1.1. In order to maximize the contact areas with the contaminated plume, PRBs are often constructed perpendicularly to the groundwater flow (Henderson & Demond, 2007). The primary removal methods could be classified into: (1) physical immobilization treatment (sorption and

precipitation); (2) chemical transformation by irreversible redox reactions; (3) biological mechanisms (Tratnyek et al., 2003).

Granular or nanosized zero-valent iron (ZVI) or iron alloys are commonly applied in abiotic PRBs for TCE dechlorination (Wilkin & Puls, 2004). ZVI reduces contaminated groundwater with chlorinated ethenes through a series of redox processes, precipitation reactions, and sorption (Henderson and Demond, 2007), and the formation of ethene would be an indicator of a successful TCE abiotic reduction (He, et al., 2008). In addition to ZVI methods, adsorption treatments are also popular in PRBs remediation technologies, methods by using materials include: granular activated carbon (GAC), bone charcoal, peat, coal, zeolite, synthetic resin, wood chips, etc. (ITRC, 2005).

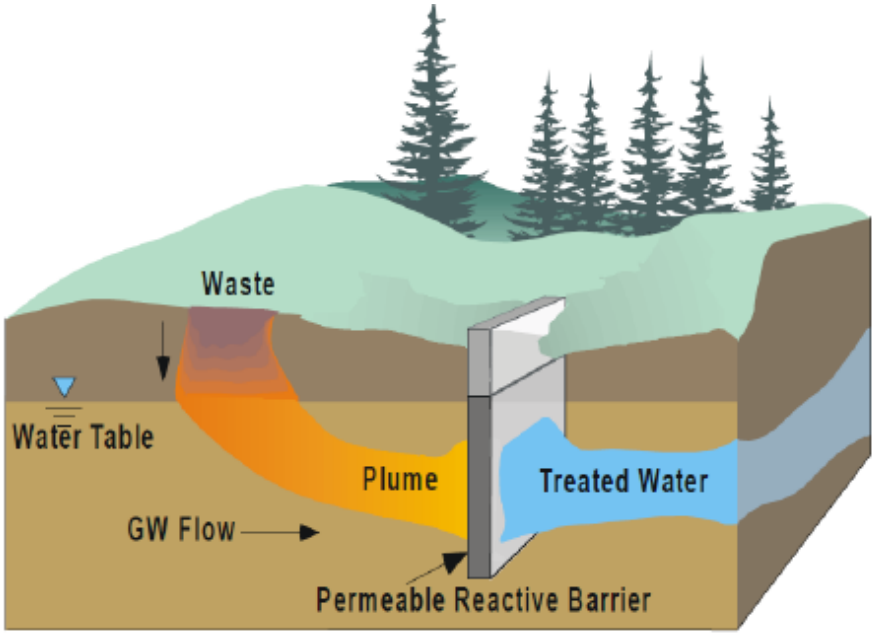


Figure 1.1 Permeable Reactive Barrier. Image from: https://www.researchgate.net/figure/A-Schematic-Diagram-of-Permeable-Reactive-Barrier_fig1_267840580

Although PRBs treatments could allow a simultaneous treatment of multiple contaminants, such as organics, nutrients, radionuclides, and heavy metals (RTDF, 2001), and does not require continuous input of energy (natural gradient of groundwater flow allows carrying pollutants

through the reactive zone) (Thiruvenkatachari, 2008), limitations to this technology do exist, including high construction costs, decreasing reactivity overtime, lengthy treatment time compared to chemical approach, and more importantly, incomplete dechlorination processes and poor dechlorination results due to plugging caused by newly-formed mineral (Shen & Wilson, 2007).

1.2.3 In Situ Remediation: Bioremediation

Bioremediation is a process to treat pollutants by altering environmental conditions to stimulate the growth of microorganisms with the ability to degrade the target contaminants. Several pilot- and full-scale field cases suggest that bioremediation is potentially more sustainable and efficient and less expensive than other remediation alternatives (EPA, 2011). Common in situ bioremediation technologies to treat chlorinated solvents include enhanced reductive dechlorination (ERD), monitored natural attenuation, and phytoremediation ((Reddy, 2008). Natural attenuation or intrinsic bioremediation means no humanistic interference factor or any activities, and bioremediation naturally occurs on its own. Enhanced reductive dechlorination requires the addition of fermentable organic substrates acting as electron donors and/or the injection of carbon sources that assist the growth of remediating microbial cultures, which is also called biostimulation (Hendrickson, 2002). Bioaugmentation, an approach that adding specific microbial strains into the contaminated region to increase the population of desired microbes which can degrade target pollutants, is also often involved in biotreatment.

Bioremediation with Permeable Reactive Barriers – Biobarriers

PRBs for bioremediation is called biobarrier or biowall. Biobarriers are currently the most promising biotechnology to dilute contaminant concentrations in groundwater plumes that occur near the surface, which are able to treat diverse types of pollutants due to their easy modification

(Reddy, 2008). Biobarriers should be constructed perpendicularly to the groundwater flow in order to best intercept the target pollutant plumes (Leeson, et al., 2004).

There are three basic types of biobarriers: active, semi-passive and passive. Active biobarriers recycles the substrate in the reactive zone for fast reaction rates and rapid removal of target pollutants. Semi-passive biobarriers need periodic injections of specific substrates to provide electrons to maintain the biological activity in the barrier system. In order to achieve rapid removal of target contaminants, substrates used in these two systems usually are readily degradable organic compounds (Cowan, 2000). Passive biobarriers demands just one-time addition of substrates, which could sustain inside biological activities over a long period (Leeson, et al., 2004).

Media or substrates for biobarriers can be classified into liquid media such as HRCTM and EVOTM, and solid media such as mulch, wood chips, and compost (Leeson, et al., 2004). These substrates, serving as electron donors, could create and sustain a reducing condition that favorable for the anaerobic transformation of chlorinated ethenes to occur within the reductive dechlorination process. Liquid substrates normally will not last very long due to flushing, and they are not able to control the plume size. Solid media not only can continuously provide electron donors and carbon sources needed for dechlorination process, but also offer a highly sorptive surface to support microbial growth. Considering the favorable market price, mulch, which can release fermentable organic compounds under an anaerobic environment underneath and also contains no toxic chemicals, is one of the most suitable solid substrates for reductive dechlorination of TCE. Mulch is shredded pieces of trees or shrubs, mainly containing cellulose and lignin. Of all mulch materials, bark mulch is the easiest to obtain and has been found to have the features to support bioremediation due to its high content of lignin (Duryea, et al., 1999). Lignin has a higher adsorption capacity than other common soil organics (Garbarini & Lion, 1986). Among bark

mulch, pine bark mulch is proved to have overall the best adsorption capacity for TCE and its daughter products (Wei & Seo, 2010).

During the bioremediation process, fermentation products (including ethanol, fatty acids, hydrogen, carbon dioxide, and methane) from the hydrolysis of pine bark mulch can support various dechlorinators including DMC and Geobacters (Shen & Wilson, 2007). Hydrolysis of mulch turns it from complex polymers into monomers, and the fermentation of monomers produce hydrogen, carbon dioxide, acetate, etc. There are some previous studies that focus on the application of mulch PRBs to TCE remediation, which will be further discussed in chapter 3.

1.3 Trichloroethene Degradation Process

1.3.1 Aerobically Oxidative Treatment

TCE degradation could be achieved through chemical oxidation processes by using permanganate, hydrogen peroxide, ozone, and activated persulfate, according to the USEPA (Huling & Pivetz, 2006). Aerobically, oxidative biological processes are cometabolic, and oxidizing bacteria such as methanotrophs need oxygen and a primary substrate such as methane and toluene to support their survival, with carbon dioxide as the end product (Wilson & Wilson, 1985). This biological mechanism could be effective when removing TCE concentration up to 1,000 to 1,200 μg/L, However, many contaminated aquifers are anaerobic and would require a continuous injection of oxygen, which could cost a lot. In addition, the solubility of oxygen could sharply decrease when TCE concentration is too high, causing a less efficient system (McCarty, et al., 1998).

1.3.2 Anaerobically Reductive Dechlorination

Reductive dechlorination involves sequential reactions: from TCE to cis-DCE to VC to ethene, wherein the chlorine atoms on the alkene molecule are replaced one by one by a hydrogen atom, which ideally ends with nontoxic ethene as the final product. Reductive dechlorination of TCE could adopt either abiotic or biotic approaches (Henderson & Demond, 2007). Compared with oxidation treatment, TCE removal in groundwater using abiotic or biotic reductive dechlorination is more favorable, resulting from the facts that TCE is highly oxidized and microbes could also gain growth benefits from the transformation.

Considering that the maximum contaminant level (MCL) of VC (an identified carcinogen) in drinking water is 2ug/L, which is even lower than TCE, incomplete dechlorination of VC as the end product might cause more serious problems (USEPA, 2009). Hence, complete reductive dechlorination is significantly critical to all remedies, no matter abiotic or biotic.

1.3.2.1 Abiotic Reductive Dechlorination

Under the abiotic condition, the reductive dechlorination via chemical reactions mainly involves the employment of ZVI or iron sulfide (FeS) (Reddy, 2008). Note that ZVI has performed the highest efficiency when reducing chlorinated compounds in groundwater (USEPA, 2002). Teerakun et al. (2008) demonstrated that the efficiency of ZVI system would not drop unless the concentration of TCE was not above 1000 mg/L. Moreover, Teerakun et al. (2011) proved that an engineered reactive barrier system combined with both abiotic and biotic processes could receive a high removal efficiency of TCE, reaching 87% with a hydraulic residence time of 26 days. However, complete reductive dechlorination was not successfully achieved: the majority of TCE daughter products were cis-DCE and VC.

1.3.2.2 Biotic Reductive Dechlorination

Biotic reductive dechlorination of TCE, a process that transforms TCE to cis-DCE to VC and finally to benign ethene as the end product, is achieved by various strains of dechlorinating bacteria such as Dhc and Geobacters. Details for dechlorinators will be presented in Section 1.5 in this chapter. Figure 1.2 demonstrates the degradation processes of certain chemicals under anaerobic environment. During this reductive anaerobic degradation pathway, every step replaces one chlorine atom with one hydrogen atom. It is noticeable that except ethene, all other chlorinated ethenes are toxic. Both TCE and VC are recognized as human carcinogens, and according to their MCL level, VC has an even higher toxicity than TCE and might cause more serious problems. Hence, complete reductive dechlorination is significantly critical to all remedies.

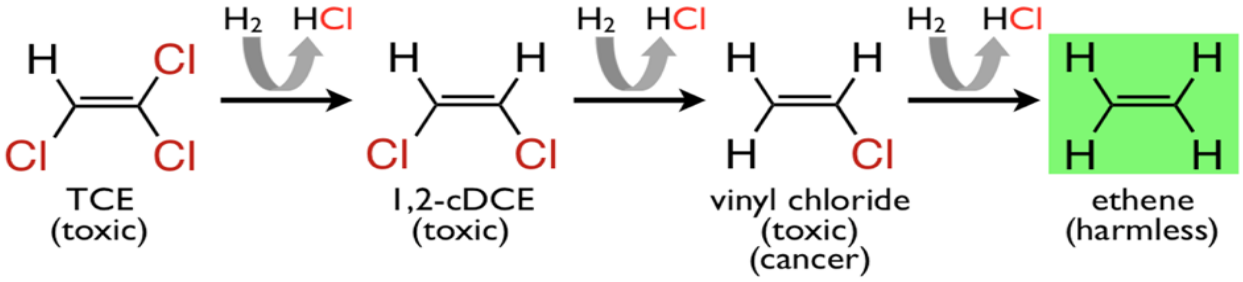


Figure 1.2 Reductive Anaerobic Degradation Pathway of Chlorinated Ethenes

1.4 Degradation Processes of Nitrate & RDX

Nitrate concentrations are an essential issue in agricultural fertilizer application and wastewater treatment: excess nitrate in waters and in drinking water supplies can cause serious algal blooms and public health problems, respectively. Under anaerobic conditions, nitrate metabolism occurs in several competing microbial processes, and nitrate reduction by microorganisms is a major biogeochemical process, which is also called denitrification. Typically

in anaerobic respiration, denitrification performed by facultative anaerobic bacteria (denitrifiers) utilizes nitrate as a terminal electron acceptor, and ultimately produces nitrogen through a respiratory electron transport chain, in response to the oxidation of an electron donor such as organic matter or hydrogen. Relative half reactions in denitrification are provided below in details:

$NO_3^- + 2\ H^+ + 2\ e^- \rightarrow NO_2^- + H_2O$ (Nitrate reductase)

$NO_2^- + 2\ H^+ + e^- \rightarrow NO + H_2O$ (Nitrite reductase)

$2\ NO + 2\ H^+ + 2\ e^- \rightarrow N_2O + H_2O$ (Nitric oxide reductase)

$N_2O + 2\ H^+ + 2\ e^- \rightarrow N_2 + H_2O$ (Nitrous oxide reductase)

The net balanced redox process in which nitrate is fully reduced to nitrogen is described below:

$2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$

Hexahydro-1,3,5-trinitro-1,3,5-triazine, commonly known as Royal Demolition eXplosive (RDX), is an explosively unstable reduced triazine-ring compound. Under anaerobic conditions, there are two common degradation routes (Hawari et al., 2000), which is presented below in Figure 1.2 and 1.3. Among products of RDX degradation, carbon dioxide and nitrous oxide are greenhouse gases. Formaldehyde is a highly toxic poison that is absorbed by inhalation.

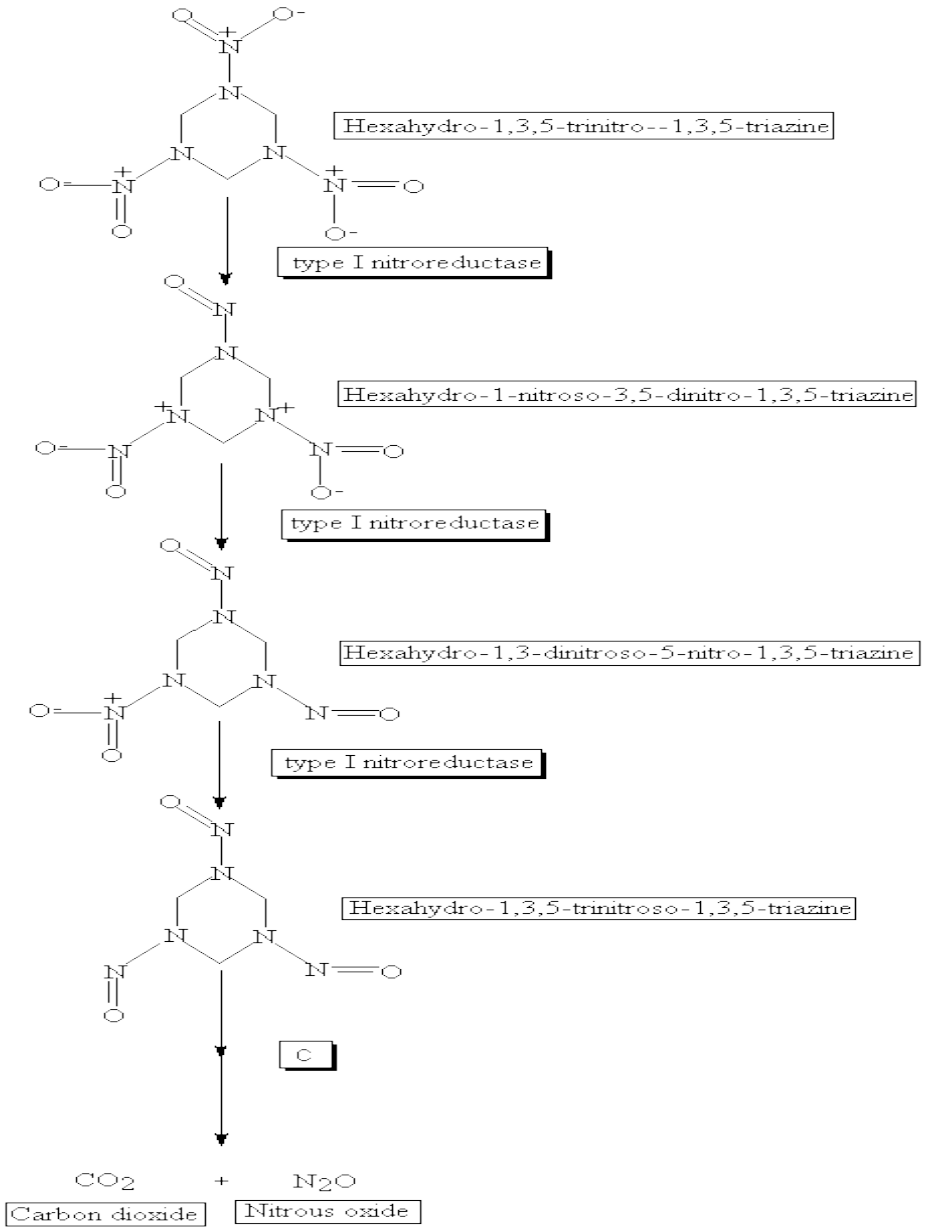


Figure 1.2 RDX Anaerobic Degradation Pathway I. Image from: http://eawag-bbd.ethz.ch/rdx2/rdx2_image_map1.html

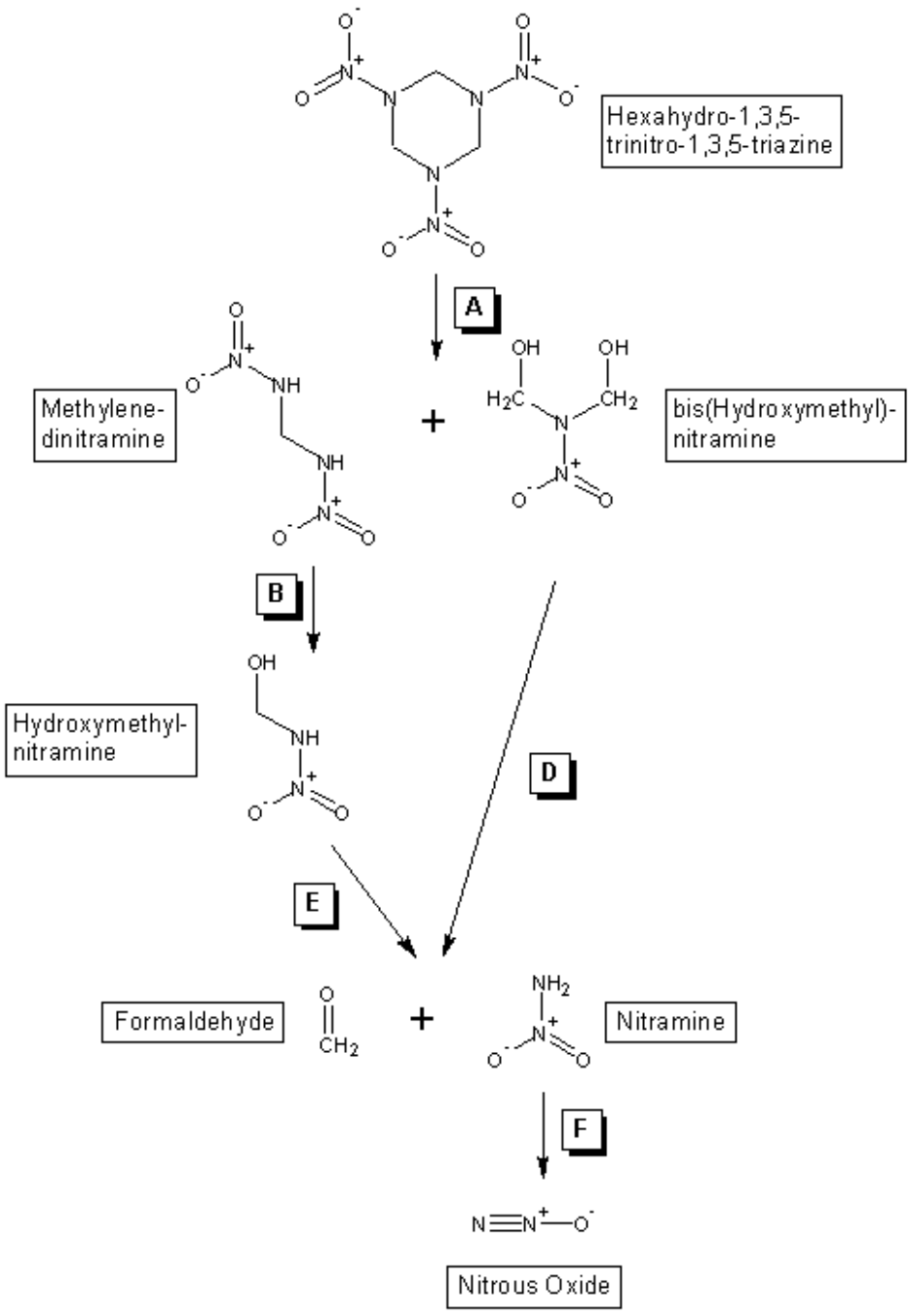


Figure 1.3 RDX Anaerobic Degradation Pathway II. Image from: http://eawag-bbd.ethz.ch/rdx2/rdx2_image_map2.html

1.5 Relevant Microorganisms

Biotic reductive dechlorination of TCE is achieved by various strains of dechlorinating bacteria. Within them, the most prominent dechlorinators are Dehalococcoides (Dhc). Among the Dhc genus, four distinct strains: Dhc mccartyi 195, Dhc sp. strain FL2, Dhc sp. strain VS, and Dhc sp. strain GT, have shown the capacity of metabolically reducing TCE to nontoxic end product ethene by anaerobic dehalorespiration itself (Maymo´- Gatell, 1999; Seshadri, 2005; Löffler, et al., 2013). Other strains excepting above four are exclusively used for DCE and VC reduction. Dhc strains are strictly anaerobic bacteria (anaerobes). However, they could still be applied to treat aerobic toxic water in mulch biobarriers if sufficient oxygen scavenging activity is present within the barrier system. A previous Cornell student named Yitian Sun conducted the research of mulch biobarriers using columns with similar setups as this thesis research. His research found that mulch near port 1 (within three centimeters of the column inlet) had efficiently reduced the dissolved oxygen (DO) level of incoming water flow and generated a qualified anaerobic environment for the occurrence of methanogenesis and dechlorination (Sun, 2014). Except for Dhc, other dechlorinators such as Geobacter, Sulfurospirillum, Desulfitobacterium, and Dehalobacter could just partially dechlorinate TCE to cis-DCE (Löffler & Edwards, 2006).

Due to Dhc strains' exclusive property, they have become essential when implementing bioremediation of chlorinated ethenes, and some mixed dechlorinating cultures have already been commercialized. Among them, the KB-1^{TM} culture from SiREM Labs of Guelph, Ontario, Canada is one the most widely used cultures in bioremediation projects worldwide. This mixed culture containing Dhc and Geobacter strains is known for its capacity of completely dechlorinating TCE to cis-DCE to VC and ultimately, to harmless ethene. Edward et al. (2014) indicated that Dhc strains in TCE-induced KB-1 culture had expressed four relevant genes: KB1_VcrA, KB1_BvcA,

KB1_TceA and KB1_RdhA5. Tang et al. (2013) demonstrated that one more gene transcript showed up as KB1_RhA1 when testing on VC-induced KB-1 culture. Geobacter strains are able to dechlorinate TCE to cis-DCE via the RDase enzyme encoded by the pceA gene.

A biomarker is a biomolecule (DNA, RNA or protein) that corresponds to a particular microbial process or state, which has been widely used to detect and quantify specific microbes. The presence and abundance of specific biomarkers corresponding to microorganisms of interest could be one of the evidence to show the existence of a living microorganism and whether expected bioremediation is occurring or not. 16S rRNA is one type of biomarkers and has been used worldwide. Hendrickson (2002) indicated that 16S rRNA gene sequences are highly conserved among Dhc microcultures and there was a significant statistical correlation between the presence of Dhc 16sS rRNA gene and the occurrence of reductive dechlorination from TCE to ethene. Note that the presence of Dhc 16S rRNA only could not lead to the conclusion that Dhc are actively conducting complete dechlorination. It is the reductive dehalogenases (RDh) in the genomes of Dhc strains which are often used as biomarkers during dechlorination ((Loffler, 2012).

RDh genes are the genes of interest for dechlorinators analysis when using molecular tools (Seshadri, 2005; Villemur, 2002). Dhc and Geobacter are two key dechlorinators in KB-1 culture. As for Dhc detection, vinyl chloride reductase genes (vcrA and bvcA) would be used as biomarkers for VC-to-ethene respiring Dhc strains, since the ultimate bioremediation goal is achieving complete dechlorination. Note that vcrA gene exists in main Dhc populations while bycA is present in minor Dhc populations. As for Geobacter detection, PCE dehalogenase (pceA) gene would be used. These primers are the same biomarkers used by previous Cornell students who also researching on KB-1TM-inoculated populations (Sun, 2014; Jin, 2016).

Methanogens are strict anaerobes which naturally exist in groundwater and surface water, producing methane under anaerobic environment. During TCE dechlorinaiton via mulch biobarriers, the survival of methanogens could serve as an indicator of an anaerobic condition in columns, and further being an indicator of the survival of Dhc (requiring anaerobic environment as well). However, methanogens would compete with Dhc for electron donors: hydrogenotrophic methanogens consume H2. When using 16S rRNA gene primers as the biomarker for methanogens, potential problems with nonspecific amplification might exist ((Luton, 2002). The methyl coenzyme M reductase (mcrA) could avoid the above risk, and it is exclusive to the methanogens and shows mostly congruent phylogeny to 16S rRNA genes (Springer, 1995). Hence, mcrA would be a better biomarker choice to analyze methanogens.

Denitrifiers and sulfate reducers, microbes which play critical roles in nitrogen cycling and sulfur cycling, also naturally occur in surface water and groundwater with excessive nitrogen fertilizer in agricultural areas. Sulfate reducers are strict anaerobes while denitrifiers prefer oxygen as an electron acceptor, but they could still reduce nitrate under anoxic environment. As for the detection of denitrifiers, in this thesis research, nitrite reductase would be selected as the biomarker for denitrifiers. This enzyme catalyzes the reduction process from nitrite to nitric oxide and is encoded by two genes: nirK and nirS (Zumft, 1997). nirS is more widely distributed in denitrifiers populations and is highly conserved (Coyne, 1989). As for the detection of sulfate reducers, dsrA is a commonly used biomarker.

1.6 Research Objectives and Hypothesis

The objectives of this thesis research are:

1) To monitor long-term column performance of mulch biobarriers with and without sulfate as an alternate electron acceptors
2) To compare the TCE-dechlorination ability of mulch biofilm communities in batch-scale microcosms in/not in the presence of RDX and/or nitrate as co-contaminants
3) To compare the denitrification process in batch-scale microcosms in/not in the presence of TCE and/or RDX
4) To detect various bacteria of interest including KB-1TM dechlorinators (Dhc and Geobacters), nitrate and sulfate reducers by using PCR amplification and DNA sequencing and to compare microbial populations along and between control and experimental columns

The hypotheses of this thesis research are:

1) Following the removal of nitrate, mulch columns would continue to effectively remove TCE over years; the presence of sulfate would have little inhibitory impact on column performance on dechlorination
2) TCE and nitrate are co-inhibitors in the process of dechlorination and denitrification
3) The presence of RDX may inhibit the dechlorination process as an additional co-inhibitor
4) Experimental columns exposed to TCE, nitrate and sulfate have higher level of sulfate reducers and denitrifiers than control columns receiving TCE only; higher populations of Geobacters and Dhc will be present in control columns

In order to explore the reductive dechlorination of TCE by KB-1TM culture-inoculated mulch biobarriers in the presence of co-contaminants including nitrate and RDX, a series of experiments have been designed and conducted:

1) Microcosms have been set up to determine TCE-dechlorination ability of KB-1TM culture in batch-scale mulch biobarriers in the presence of nitrate and RDX
2) Column-scale experiments have been conducted to explore TCE-dechlorination ability of KB-1TM culture in mulch columns in the presence of sulfate as an electron acceptor.
3) 16 DNA samples in total for DNA sequencing analysis

This thesis research will use pine bark mulch filled columns/microcosms and KB-1TM culture inoculum to treat TCE-contaminated oxygenated tap water. The dechlorination process and nitrate concentration will be monitored along with time.

Chapter II Literature Review

2.1 Trichloroethene & RDX Properties, Occurrences in Nature and Routes of Uptake

TCE is a clear colorless volatile liquid having a chloroform-like odor. TCE is non-combustible with a low-boiling point, denser than water and slightly soluble in water. The natural occurrence of TCE has shown up in one red microalga and in temperate, subtropical and tropical algae (IARC, 1995). TCE has a low tendency to bioaccumulate in the food chain and enters the human body through air breathing, direct skin/eye contact, and water drinking.

RDX is a solid organic compound, which is white without any smell or taste, widely used as an explosive in World War II (more energetic than TNT) and remains common in military applications. It is chemically classified as a nitramide and its chemical properties are similar to HMX. It is a synthetic product, thus it does not naturally occur in the environment. RDX has a relatively low potential of bioconcentrating in aquatic organisms, due to its low octanol-water partition coefficient and low experimental bioconcentration factor (ATSDR, 2012). CRREL (2006) indicated that RDX was possible to bioaccumulate in plants, which could be a potential exposure route to herbivorous wildlife.

2.2 Toxicity, Mobilization Routes, Exposure and Threat of Chlorinated Ethenes & RDX

2.2.1 TCE

The Maximum Contaminant Level (MCL) for TCE in drinking water is 0.005 mg/L, for cis-DCE and trans-DCE is 0.07 mg/L and 0.1 mg/L, respectively. However, the MCL for VC is only 0.002 mg/L (USEPA, 2008), indicating an even higher toxicity than TCE. Both TCE and VC are recognized as human carcinogens.

In the atmosphere, TCE is converted to hydroxyl radicals by photochemistry reaction (Singh, et al., 1982). In surface water, TCE tends to spread rapidly into the atmosphere due to the facts biodegradation and/or hydrolysis would occur at a low rate (ATSDR, 1997). In the soil, TCE will displace soil pore water and continue to sink until it reaches an impermeable layer such as clay (ATSDR, 1997). TCE has been found to have a medium-to-high mobility in soil and high mobility in sandy soil (Wilson, et al., 1981). TCE has a high volatility due to its high Henry's Law Constant.

Routes of TCE exposure include inhalation, skin/eye contact, and ingestion. As for inhalation, TCE vapor is readily absorbed from the lungs. As for skin/eye contact, skin irritation and minor corneal injury may occur when exposing to liquid TCE. As for ingestion, it can result in severe CNS depression due to rapid and substantial gastrointestinal absorption. Note that children are more vulnerable to toxic TCE than adults (USEPA, 2008).

According to the National Priorities List (NPL), TCE has been found in 852 of the most severe hazardous-waste polluted sites, out of 1430 in total (ATSDR, 2003). Due to its mass production and large amounts of incidences of uncontrolled release, TCE is also reported to be one of the biggest environmental risk drivers in the world (Moran, et al., 2007). To date, there is no antidote for TCE poisoning. Medical treatment consists of support of respiratory and cardiovascular functions.

2.2.2 RDX

Oral toxicity of RDX depends on its physical status: the lethal dose (LD50) was 100 mg/kg for finely powdered RDX, and 300 mg/kg for coarsely granular RDX (Schneider et al., 1977). The MCL for RDX in drinking water is 0.00061mg/L (Abadin et al., 2012). This substance has been assigned a low to moderate toxicity with a classification of possible human carcinogen (USEPA,

1993) and further research is ongoing: RDX is highly possible to be reclassified into more serious toxicant (Smith et al., 2007).

The most likely route of exposure to RDX at/near hazardous waste sites is the injection of contaminated drinking water or crops, except other potential exposure routes such as dermal contact or inhalation (ATSDR, 2012). When overexposed to RDX through inhalation or ingestion, RDX will target humans' nervous systems and cause potential symptoms including headache, dizziness, vomiting, tremor, etc. (HSDB, 2013). It may cause even more severe health problems such as liver and kidney damage (ATSDR, 2012).

RDX is likely to be released to the environment through spills, open incineration of munitions, detonation /disposal of ordnance, and munitions processing and manufacturing facilities (ATSDR 2012). According to EPA NPL, RDX had been detected at more than 30 sites by 2007 (HazDat, 2007). In the atmosphere, RDX will exist in a particulate phase and settle by dry or wet deposition, which is then easily exposed to surrounding communities (HSDB, 2013). RDX does not tend to be retained by most soils due to its low soil sorption coefficient value, i.e., RDX can easily migrate to groundwater and flow through the vadose zone, contaminating the underlying groundwater aquifers (CRREL, 2006).

Chapter III Previous Relevant Research

3.1 Former Column Research on Dechlorination

PRBs inoculated with a population that able to conduct reductive dechlorination population (Dhc strain) has been confirmed to effectively remediate PCE plumes (Lendvay, 2003). Since mulch was chosen to be filled into the PRBs in this research, previous relevant studies that also focuses on the application of mulch-filled PRBs to TCE remediation could provide some references.

Shen and Wilson (2007) found that TCE removal rates in mulch columns with hematite and limestone were higher than that in mulch columns with sand, however, none of them was bioaugmented. As a result, after 793 days of operation, less than 1% of TCE removal was given rise to the biological reductive dechlorination: 80% to 90% of TCE removal was due to the abiotic transformation by FeS minerals formed in local groundwater. The daughter products from biological reduction dechlorination would be cis-DCE, VC, and ethene, while the main product of abiotic FeS-based TCE reduction was acetylene.

Shen et al.'s follow-up study (Shen et al., 2010) found that, when columns and operational conditions remained the same but with bioaugmentation, i.e., inoculation of an enrichment culture of dechlorinating bacteria, complete transformation of TCE to ethene was achieved using plant mulch as the electron donor. Kinetic analysis of the methane production indicated that the plant mulch biobarriers are able to sustain a long-term biological activity for 10 years before replacing new mulch or adding new electron donors.

Lu et al. (2008) examined the performance of a pilot-scale mulch biobarriers under natural attenuation with detected Dhc. After one month of operation, results turned out that degradation

from TCE to VC succeed but there was no production of ethene, even though Dhc DNA was present in the area of mulch biobarriers.

Oztürk, et al. (2012) achieved a complete reductive dechlorination of TCE to ethene on an up-flow eucalyptus mulch column with a bioaugmentation with a TCE-degrading enrichment culture. The Dehalococcoides population was stated as 1.21 x 108 cells/μg mulch sample. However, no quantified data was acquired due to the instrument detection limit. They finally achieved a 74% efficiency of dechlorination process from TCE to ethene.

3.2 Former Research on Inhibition Impact on Dechlorination

Nelson et al (2002) discovered that PCE dechlorination in a hydrogen-fed reactor was inhibited in the presence of nitrate and sulfate. However, they did not clarify whether nitrate and/or sulfate and/or daughter products of them was responsible for this inhibition. In addition, extremely slow reduction rates of nitrate were observed in the 1% H2-fed reactor under an H2-limited condition.

Heimann et al (2005) found out that sulfate concentration at 2.5 mM would limit microbial dechlorination when hydrogen supply was limited. Conversely, sulfate did not affect dechlorination when hydrogen was adequate. Thus, whether the limitation was attributed to competition for electron donor or other mechanisms was not clarified. Berggren et al (2013) indicated that a decline in dechlorination performance could also result from a shift in the microbial community, which may be related to competition for hydrogen at low concentrations, or toxicity/inhibition effects from daughter products of sulfate reduction, especially sulfide. Still, the specific mechanism remains uncertain.

Relevant research believed that nitrate had no inhibition on PCE and TCE dechlorination in axenic cultures of Dhc strain FL2 and Geobacter strain SZ (Sung et al., 2006; He, et al., 2005). And Recently, Yin et al (2019) demonstrated that the daughter product of nitrate reduction, nitrous oxide (N2O), decreased dechlorination rates at its low micromolar concentration and caused an incomplete dechlorination of PCE in Geobacter lovleyi strain SZ and of cDCE and VC in Dhc mccartyi strain BAV1 axenic cultures.

According to Ahmad et al (2008), complete removal of 90 ppb level of influent RDX in steady-state pine mulch column effluent was successfully achieved at bench scale and no binding of RDX to the mulch was observed. Ahmad et al (2009) further achieved a larger than 93% RDX removal in a sustained mulch PRB, with no accumulation of toxic intermediates from RDX degradation. indicating the potential of applying organic mulch biobarrier to RDX degradation at pilot scale in practice. Since mixtures of explosives and chlorinated solvents have occurred in groundwater at several sites in the United States, Young et al (2006) created experiments to test whether two specific microbial cultures (anaerobic sludge and a facultative enrichment culture) were able to biodegrade dual-contaminant mixtures of TCE and RDX under anaerobic condition. Result turned out that both cultures successfully biodegraded mixtures of RDX and TCE.

3.3 Former Relevant Research Conducted by Past Cornell Students

Yitian Sun (2014) proved that the mulch columns provided a good habitat for dechlorinators including both Dhc strains and Geobacter at least 5 months after inoculation. He also achieved a 73% to 99% complete reductive dechlorination of oxygenated tap water contaminated with 1 mg/L of TCE on the mulch biobarriers, with an HRT of ~3 days, which was inoculated with 1:1000 KB-1^{TM} enrichment culture.

Ye Jin (2016) found that dechlorination by KB-1^{TM} inoculated mulch biobarriers in experimental columns did not happen when electron acceptors nitrate and sulfate were present at 1 mM, and only partial dechlorination was shown when nitrate and sulfate were reduced to 0.25 mM. While complete dechlorination was seen in control columns within 200 days of inoculation at 1:100 dilution into the column pore water.

However, the limitation/competition mechanisms is still unclear. Potential impacts of other alternative electron acceptors such RDX is also unknown. Thus, further research is ongoing.

3.3.1 TCE and cis-DCE Sorption Assays

Sun (2014) determined the sorption capacity of pine bark mulch for TCE and cis-DCE via batch-scale experiments. Results show that Kf (the Freundlich isotherm constant, L/kg) for the cis-DCE adsorption isotherm (36.4 L/kg) is greater than that of TCE (16.8 L/kg), thus the adsorption tendency for cis-DCE is greater than that of TCE, meaning that cis-DCE is more soluble than TCE in water with a higher polarity index and has less affinity for low-polarity organic material such as mulch.

3.3.2 Former PCR/qPCR Data for mcrA and vcrA genes

End-Point Polymerase Chain Reaction (PCR) amplification of target genes is carried out on the Eppendorf Mastercycler Gradient ThermoCycler. Different primers and PCR programs are applied to detect different microbial groups. Quantitative Polymerase Chain Reaction (qPCR) is applied to quantify the genes of interest.

The mcrA gene is used to be the biomarker for methanogens and the vcrA gene is used to be the biomarker for the main Dhc populations. Note that the vcrA gene can be used specifically as the biomarker for VC-to-ethene respiring Dhc strains.

Sun (2014) confirmed the existence of the main Dhc populations in the columns from PCR tests on vcrA: PCR results were good and best match from BLAST was Dehalococcoide s sp. KB1 vcrA gene. No PCR was conducted for mcrA and no qPCR was carried out for neither mcrA nor vcrA.

Jin (2016) run PCR programs on both mcrA and vcrA, and a summary of PCR results is provided in Table 3.1 below.

Table 3.1 PCR results for mcrA and vcrA

lane #	Taking Sample Date	Description	Expected Amplicon Length (bps)	Positive or not (Y/N)	Expected PCR products or not (Y/N)
2	2/15/2016	C1P2, *mcrA*	490	Y	Y
3	2/15/2016	C2P2, *mcrA*	490	Y	Y
4	2/15/2016	C3P2, *mcrA*	490	Y	Y
5	2/15/2016	C4P2, *mcrA*	490	Y	Y
6	2/15/2016	C1P6, *mcrA*	490	Y	Y
7	2/15/2016	C2P6, *mcrA*	490	Y	Y
8	2/15/2016	C3P6, *mcrA*	490	Y	Y
9	2/15/2016	C4P6, *mcrA*	490	Y	Y
10	10/19/2015	C1P2, *mcrA*	490	Y	Y
11	10/19/2015	C2P2, *mcrA*	490	Y	Y
12	10/19/2015	C3P2, *mcrA*	490	Y	Y
13	10/19/2015	C4P2, *mcrA*	490	Y	Y
14	11/24/2015	C1P2, v*crA*	441	Y	Y
15	11/24/2015	C2P2, v*crA*	441	Y	Y
16	11/24/2015	C3P2, v*crA*	441	Y	Y
17	11/24/2015	C4P2, v*crA*	441	Y	Y
18	11/24/2015	C1P6, v*crA*	441	Y	Y
19	11/24/2015	C2P6, v*crA*	441	Y	Y
20	11/24/2015	C3P6, v*crA*	441	Y	Y

21	11/24/2015	C4P6, v*crA*	441	Y	Y

Therefore, the presence of Dhc and methanogens could be confirmed in every column, port 2 and port 6.

In addition, qPCR was performed with primers for mcrA and vcrA to quantify methanogens and Dhc strains in the mulch column porewater. qPCR targeting the mcrA gene was performed with mlas F as the forward primer and mcrA R as the reverse primer. qPCR targeting the vcrA gene was performed with RDh A14 642F as the forward primer and RDhA14 846R as the reverse primers. Results turned out that the populations of Dhc are smaller in experimental columns than that in control columns, but for methanogens, the populations are indistinguishable in control and experimental columns.

Chapter IV Materials and Methodology

4.1 Chemical Reagents

TCE (99.5%, Fisher Scientific) was adopted to prepare saturated TCE stock solution and TCE standards. Cis-1,2-DCE (97%, Sigma-Aldrich) and was used to prepare cis-DCE standards. RDX (1000 mg/L, RESTEK CORP 31666) was used to prepare RDX stock solution. Potassium sulfate (Fisher Scientific, certified A.C.S.) was added into the tap-water reservoir. Potassium nitrate (Fisher Scientific, certified A.C.S.) was added into microcosms. High purity compressed nitrogen (Airgas), ultra-high purity hydrogen (Airgas), and compressed air (Airgas) were used as a carrier flow in Gas Chromatography (GC) with Flame Ionization Detection (FID). High purity compressed nitrogen (Airgas) was also injected in microcosms to sustain an anaerobic environment.

4.2 Mulch Column System Setup

On January 15, 2015, four glass columns (5-cm diameter, 60-cm height) were chosen and set up to simulate the mulch biobarriers receiving TCE-contaminated groundwater. Each column is supposed to have seven sampling ports from the bottom up, with 8-cm spacing between adjacent ports 1, 2, 3 and 4, and 10-cm spacing between ports 4, 5, 6 and 7. And there are 2-cm heights of mulch locating below port 1 and above port 7 (Sun, 2014). A sketch of column systems is presented in Figure 4.1 below. Note that experimental column 4 lost port 7 due to a crack on its top during the summer of 2015. Column 1 and column 2 are replicate as control columns, and receive only aerobic tap water (DO of around 8 mg/L, pH between 6.5 and 7.5) pumped in. Column 3 and column 4 are replicate as experimental columns, and receive aerobic tap water containing sulfate with a concentration of 0.25mM. Two liquid reservoirs were set up to hold the liquid pumped into

each column. A peristaltic pump acts as a flow-rate controller to allow the liquid flowing from the reservoirs and along the column from the bottom up with a flow rate of 0.2 mL/min (288 mL/day). Based on the porosity of packed columns and the flow rate, the Hydraulic Residence Time (HRT) is estimated to be 2.69 days. TCE with a concentration of 1 mg/L is pumped into each column from the bottom via four syringe pumps. Hence, the bottom part of each column mimics the front face of a biobarrier and the top part acts as the rear face. Relevant column operation and sampling are conducted under room temperature of 22 ℃.

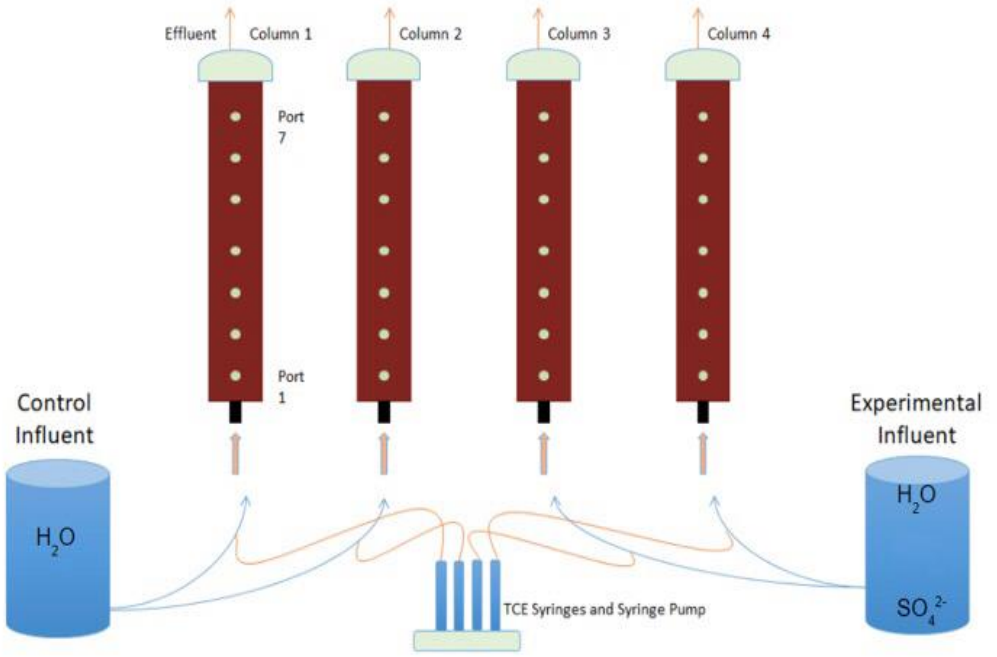


Figure 4.1 Sketch of mulch columns setup. Experimental columns had sulfate in influent. Tap water flow direction is upward with a retention time of 2.69 days.

Pine bark mulch (Agway in Ithaca, NY) was added into four columns as the medium for bioremediation and serves as the electron donor. Limestone chips (Fisher Scientific Cat. S25201A), applied as 40% by weight of dry mulch, were also added in each column to offer a buffer and avoid excessively low pH caused by acid production (organic acid and carbon dioxide) by fermentation

of organics in the mulch. KB-1^{TM} culture was inoculated into each column via sterile syringe at a dilution of 1:100 to achieve a total inoculum of approximately 10^9 *Dhc* cells (Waller, 2005).

4.3 Microcosms Setup

In order to compare the TCE-dechlorination ability of KB-1TM culture in batch-scale mulch biobarriers in/not in the presence of nitrate and RDX as co-contaminants, 12 batch-scale microcosms were set up on February 3, 2019 (day 1525 since column setup) using 12 volume of 160 mL glass bottles. Each microcosm had 50 mL of liquid (tap water after purging with nitrogen) and 110 mL of headspace (nitrogen) and was classified into three groups X, Y, Z. Figure 4.2 below illustrates the details.

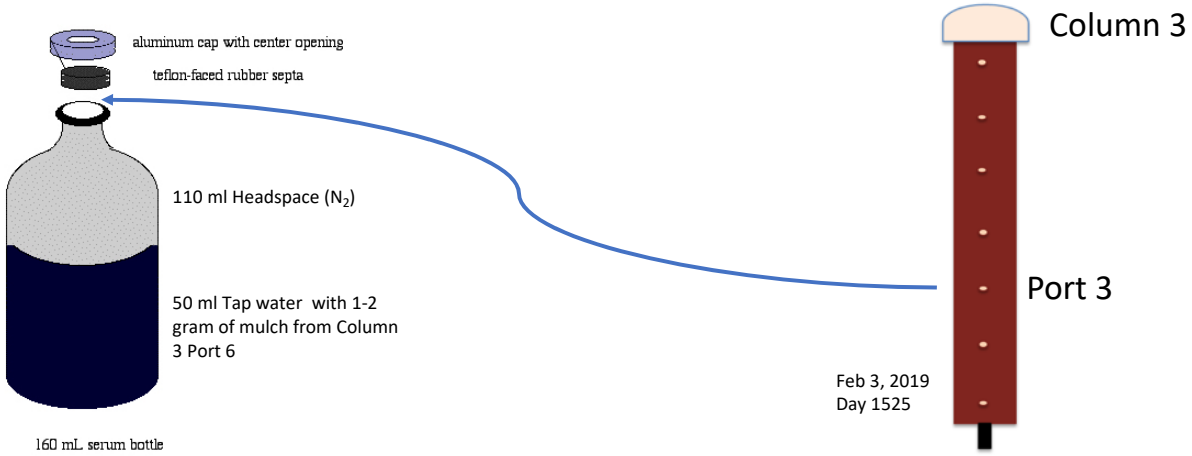


Figure 4.2 Sketch of microcosms setup.

During the early stage of this research, Group X had three replicate X-A, X-B, X-C with approximately 1 gram of wet mulch and 1 mg/L of TCE added, and had a control bottle with only 1 mg/L of TCE injected. Group Y had three replicate Y-A, Y-B, Y-C with 1 gram of wet mulch and 1 mM of nitrate added, and had a control bottle with only 1 mM of nitrate injected. Group Z had three replicate Z-A, Z-B, Z-C with 1 gram of wet mulch, 1 mg/L of TCE, and 1mM of nitrate

added, and had a control bottle with both 1 mg/L of TCE and 1 mM of nitrate injected. Note that a certain amount of extra electron donor (butyric acid) was injected into each microcosm except control groups to activate anaerobes living in.

During the later stage, 1 mg/L of RDX was respectively injected into X-C, Y-C, and Z-C, with other settings remaining the same as before.

All mulch used was taken out by tweezer from column 1 port 3, with nitrogen flushing during the whole sampling process to avoid oxygen invading into the column. Later weighting and distribution of mulch into 12 microcosms was carried out in an anaerobic chamber glove box. The average water content of wet mulch was found to be 95%. All sampling and operations were conducted under room temperature. To avoid the crystallization of ions and to increase the surface contact, all microcosms stayed shaking on a platform shaker (Innova 2000 Platform Shaker) with a rate of 150 RPM, when there was no sampling needed.

4.4 Gas Chromatography

TCE, cis-DCE, VC, ethene, methane in each column are measured and monitored on a Perkin Elmer Autosystem Gas Chromatography (GC) with Flame Ionization Detector (FID). Sampling procedures as described in Ye Jin (2016). detection limits and setting details are shown in Appendix A.

The experiment to determine new PH/PA ratios (Peak Height / Peak Area) of each dechlorination product was conducted to achieve a more reasonable and precise mass balance, with details presented in Appendix B.

Calibration curves (relationship between the GC peak height and concentration) of TCE and cis-DCE were built using a series of standards (with known concentrations) prepared from stock

solutions, and are shown in Appendix C. Since there is no pure VC and ethene reagent in the lab during the period of this research to calibrate GC readings for VC and ethene, estimated calibration factors for these two products was determined, with details presented in Appendix D.

4.5 Nitrate/Nitrite Assay

A simple, rapid spectrophotometric method for simultaneous evaluation of nitrate and nitrite concentrations in a microtiter plate format was developed by Miranda et al. (2001) and is employed in this research. The principle of this nitrate/nitrite assay is the reduction of nitrate by vanadium(III) combined with detection by Griess reaction. This assay is sensitive to the concentration of 50 uM. Stock reagents needed include nitrate standard stock, vanadium chloride (VaCl3), (N-(1-Napthyl) ethylenediamine dihydrochloride (NEDD), and sulphanilamide (FREDD). For blanks, replace VaCl3 with DI water. 15 to 90 minutes' incubation in the dark under an ideal room temperature is required. When a hot pink color develops, it is the time to measure. Measure absorbance of standards on the spec at 540 nm.

As for nitrate and nitrite measurement, 250 uL liquid samples were taken from each microcosm per measurement and went through the process of centrifugation (Sorvall Legend Micro 21R Centrifuge) under specific conditions (speed of 7,000 RPM, 6 minutes), in order to sink dissolved mulch particles. Then 100 uL supernatant would be taken out for later nitrate measurement and another 100 uL supernatant would be taken out for later nitrite measurement.

Tecan Infinite Plate Reader (Infinite 200 PRO) and Magellan software were applied to measure the absorbance of nitrate/nitrite standards and samples. Measure the absorbance of standards and samples on the spec at 540 nm.

4.6 PCR Amplification and DNA Sequencing

Polymerase chain reaction (PCR) is a method widely used in molecular biology to generate thousands to millions of copies of a particular DNA segment of interest. DNA that exponentially amplified by PCR may be sent for sequencing.

In this research, 16 DNA samples, extracted from port 2 and port 6 on four columns by former MS student Ye Jin, was amplified via PCR method and then sent out for sequencing (PCR amplification was conducted for 16S rRNA genes). 16S rRNA gene amplicon Illumina sequencing was determined by Sanger sequencing, Cornell DNA Sequencing Facility. MiSeq 2x300 bp was applied for senquencing: 300 forward and 300 reverse reads (amplicons we have were 582 bases). QIIME 2™, a next-generation microbiome bioinformatics platform, and R Studios were further applied in data visualization to analyze microbial abundance and distributions along the columns.

Chapter V Results and Discussion

5.1 Column Performance

GC-FID was applied to compare methane production and quantify dechlorination ability along and across control and experimental columns. Timeline of the operational stages of columns is shown in Figure 5.1 and Table 5.1.

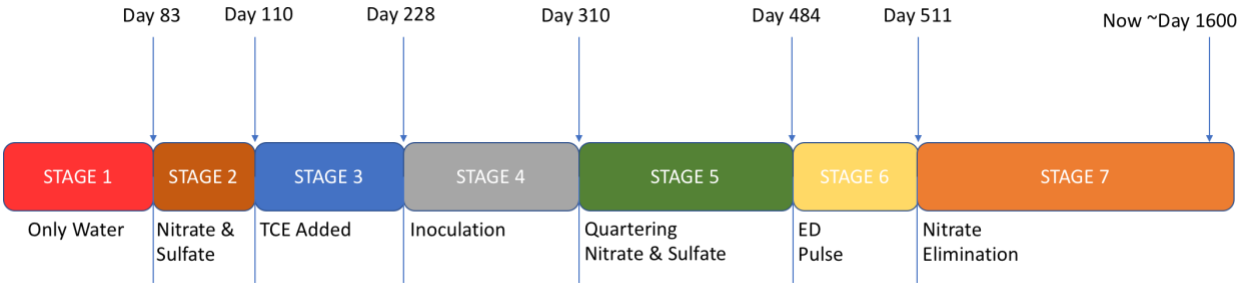


Figure 5.1 Timeline of the operational stages of columns

Table 5.1 Timeline of column operations

Date & Day	Operation
1/15/2015 day 0	Columns set up
2/15/2015 day 30	Water only started to flow through all columns
4/2/2015 day 83	1 mM Nitrate and 1 mM sulfate started to flow through all columns
4/7/2015 day 110	1mg/L TCE started to flow through all columns
8/3/2015 day 228	Inoculated all columns with KB-1 mixed culture
10/25/2015 day 310	Nitrate and Sulfate dosage quartered
12/1/2015 day 347	DNA extracted from port 2 and 6 on all columns for further DNA sequencing analysis
1/31/2016 day 408	DNA extracted from port 2 and 6 on all columns for further DNA sequencing analysis
4/16/2016 day 484	Electron donor pulse (butyric acid)
5/13/2016 day 511	Nitrate Elimination

5.1.1 Concentration of Chlorinated Ethenes in Columns

Column performances were continuously monitored every two/three weeks from May 2018 to March 2019. Two typical days (Sep 28, 2018, day 1381; Feb 15, 2019, day 1537) were selected out to show the results of column performances and concentration profiles of chlorinated ethenes between columns. For each day, column 1 & 2 (control columns without sulfate added) and column 3 & 4 (experimental columns with 0.25 mM sulfate flowing through) were chosen to be compared (Figure 5.2 & 5.3).

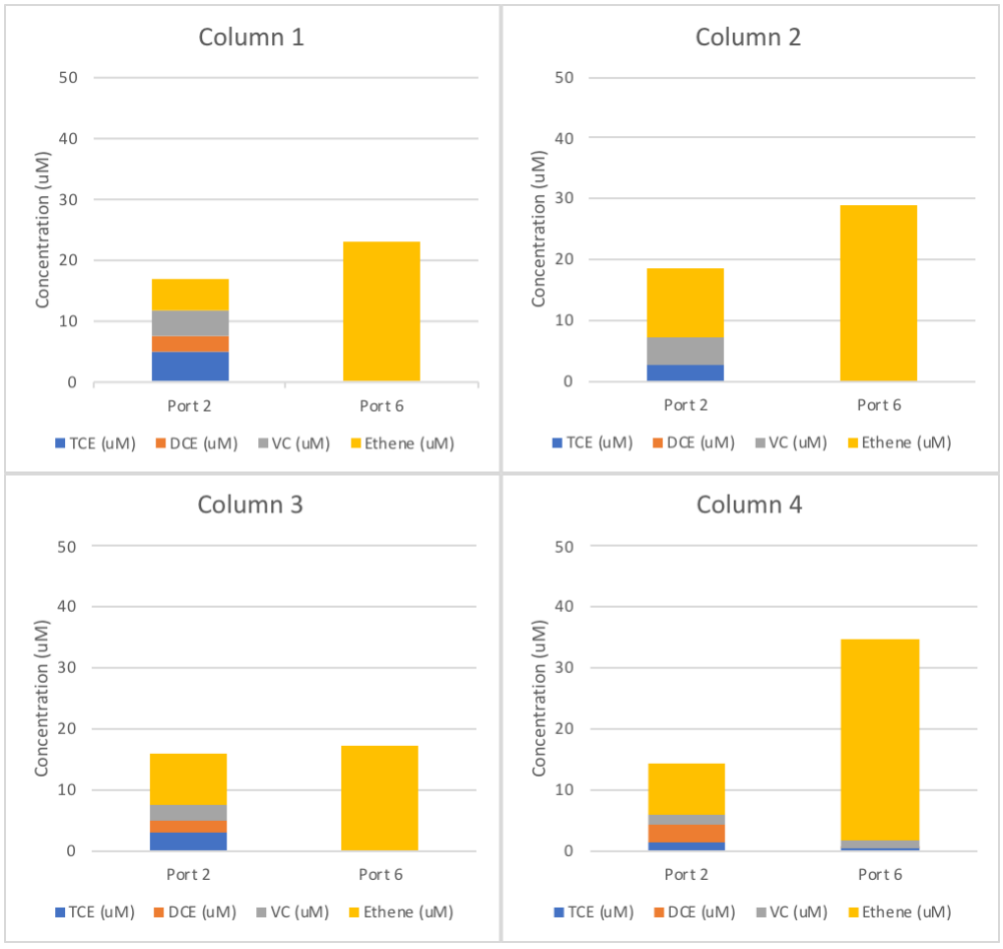


Figure 5.2. Concentrations at port 2 and port 6 of different ethenes on four columns. Measured on Sep 28, 2018 (Day 1381 since mulch columns setup). Experimental columns (column 3 & 4, bottom) had sulfate in influent.

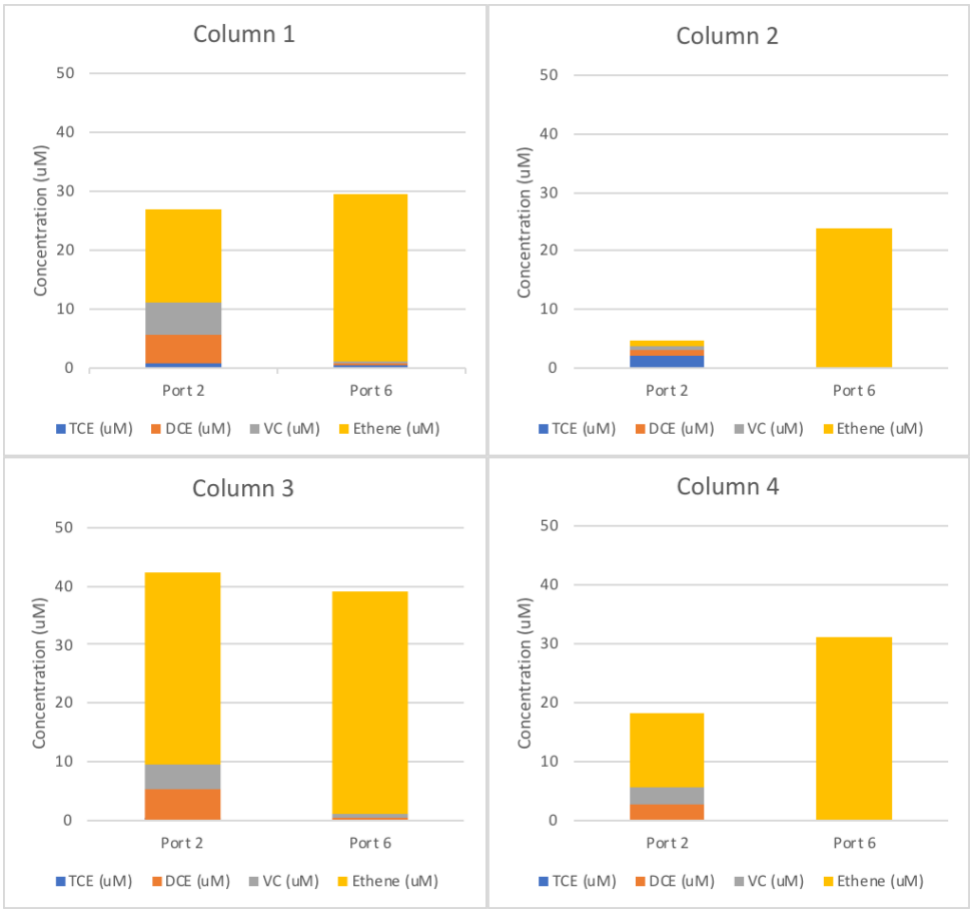


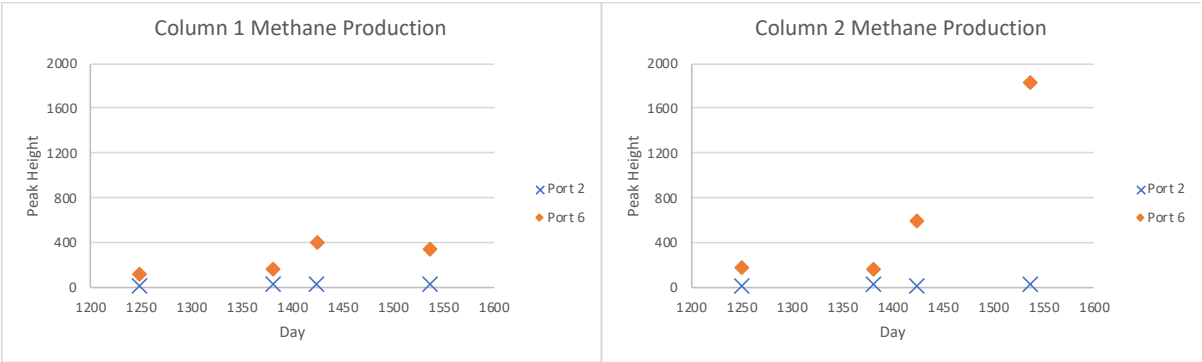
Figure 5.3 Concentrations at port 2 and port 6 of different ethenes on four columns. Measured on Feb 15, 2019 (Day 1537 since mulch columns setup). Experimental columns (column 3 & 4, bottom) had sulfate in influent.

It was observed that the concentration of TCE decreased from port 2 to port 6 from the bottom up on every column, and there was only ethene or ethene with the largest proportion at port 6 on all columns, which proves that all the columns achieved a complete dechlorination. It is noticeable that the total chlorinated ethenes were higher than 7.6 uM of TCE that was pumped in, especially for ethene. Reasons could be the sorption capacity of pine bark mulch or glass wall for these chlorinated ethenes. Clogging inside the column system due to long-time operation might also be a factor. In addition, when looking into the concentrations of TCE and its daughter products at port 2 or port 6, there was no significant difference between control and experimental columns: all four

columns had similar performance. These show that mulch biobarrers are capable of conducting complete dechlorination, and, there is no significant inhibition impact of adding sulfate at 250 μM on mulch biobarriers' ability to fully dechlorinate 7.6 μM TCE over years of performance.

5.1.2 Methane Production

Methanogens living in the columns could produce methane from consuming carbon dioxide and hydrogen or from acetate under anaerobic environment. These strict anaerobes not only came from the KB-1 TM mixed culture but also are native to the mulch. The formation of methane could serve as an indicator of a favorable anaerobic environment for dechlorinators including Dhc and Geobacters. Methane production at port 2 and port 6 along the columns were continuously monitored every two/three weeks from May 2018 to March 2019. Four typical days from 2018 to 2019 were selected out to show the results of methane production along the columns and methanogenesis trends between control and experimental columns. Column 1 and column 2 are control columns without sulfate added; column 3 and column 4 are experimental column with 0.25 mM sulfate flowing through (Figure 5.4).



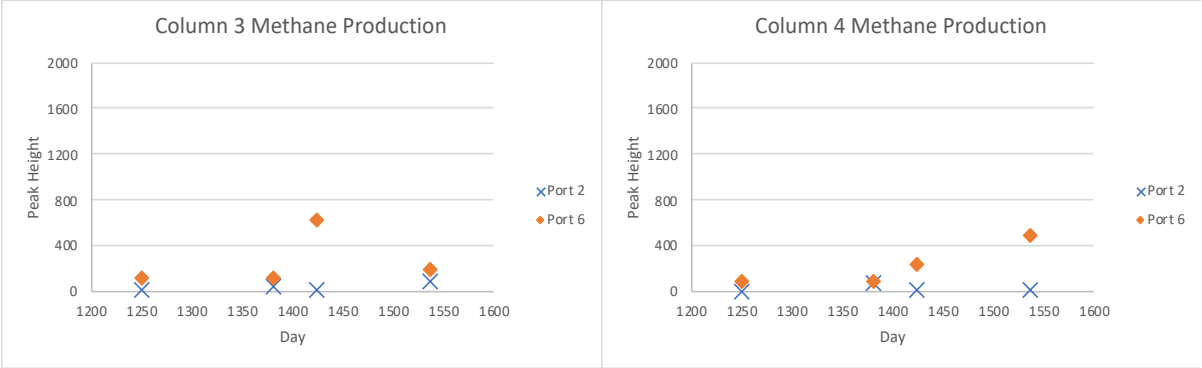


Figure 5.4 Methane Production in four columns from Day 1250, Day 1381, Day 1424, and Day 1537 since mulch columns setup (May 20, 2018; Sep 28, 2018; Nov 10, 2018; Feb 15, 2019). Experimental columns (column 3 & 4, bottom) had sulfate in influent.

It can be seen that higher amount of methane was produced at port 6 than port 2 on every column, which means that more methanogens were accumulated near the end boundary of the mulch biobarriers.

5.2 Microcosm Performance

5.2.1 Mass Balance of Chlorinated Ethenes in Batch Reactors

Microcosms were created on Feb 3, 2019 (Day 1525 since mulch columns setup) using column mulch including its corresponding biofilm community. Twelve microcosms were created anaerobically and separated into three groups: group 1 has three replicates A, B, C with 1 mg/L TCE and one control without mulch; group 2 has three replicates A, B, C with 1 mM nitrate and one control without mulch; group 3 has three replicates A, B, C with both 1 mg/L TCE and 1 mM nitrate and one control without mulch. Note that 1 mg/L RDX was injected in Replicate C in each group during the second period of the microcosm experiment. RDX solution was not applied into any batch reactors during the first period of the microcosm experiment.

According to the GC results, degradation of TCE did not occur in any microcosms during the first 12 days before any extra electron donor was added. Reasons for this stagnation may be as

follows: the anaerobic chamber that we used to set up the microcosms was not perfectly anaerobic at that moment, since later measurements showed the oxygen sensor was giving false readings, and nonnegligible amount of oxygen may be uncontrollably leaked into microcosms, inhibiting the activities of anaerobes of interest in the microcultures until anaerobic conditions again established themselves. Therefore, in order to activate the anaerobes inside, 6 meeq/L of the fermentable electron donor, butyric acid (conversion factor 20 meeq/mmol butyrate), was injected into each microcosm instead of control groups. Details for calculation are provided in Appendix E.

Figure 5.5 and Figure 5.6 illustrate the mass balance of chlorinated ethenes in batch-scale microcosms during two experimental periods. On each graph, day zero represents the first day of adding butyric acid instead of first day of adding mulch (as noted we believe there was oxygen in the microcosms at setup). Day 32 (indicated with a black line in Figure 5.5, etc.) was the first day of period two after purging. Because the performance of replicate A, B and C in each group are similar to each other, replicate A and C are selected out in each group to better present and compare. Concentrations data of all replicates will be provided in Appendix F. Note that TCE-control in light blue color on Figure 5.5 & 5.6 represents the abiotic control with TCE.

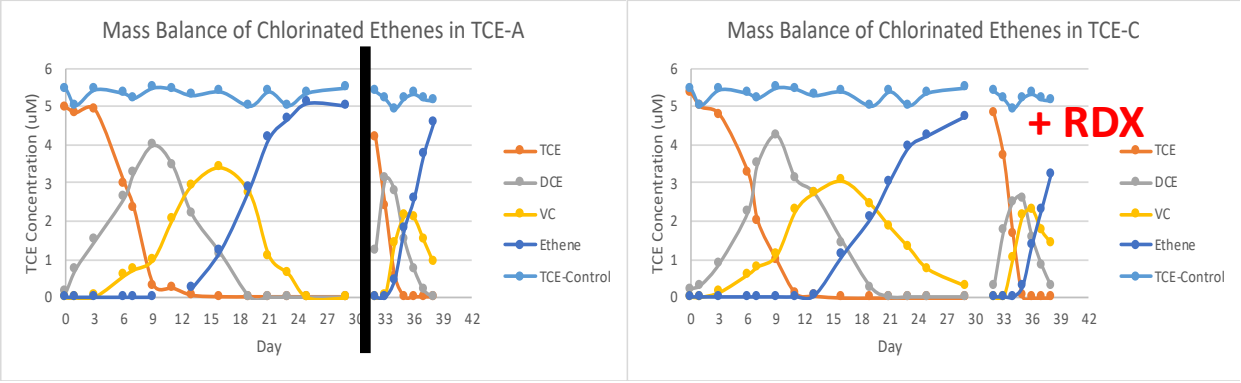


Figure 5.5 Mass balance of chlorinated ethenes in microcosms with TCE only (replicate A, left and replicate C, right). 1 mg/L RDX was injected into replicate C at the beginning of period two.

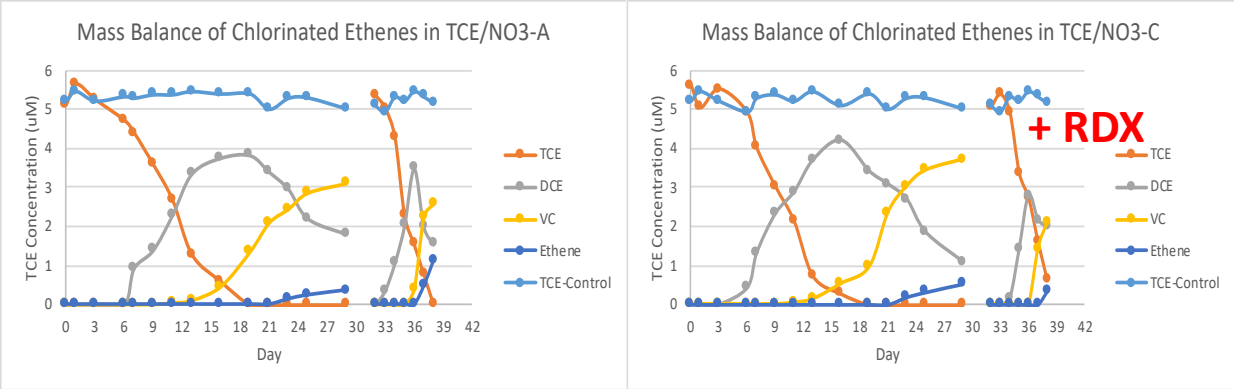


Figure 5.6 Mass balance of chlorinated ethenes in microcosms with both TCE and nitrate (replicate A, left and replicate C, right). 1 mg/L RDX was injected into replicate C at the beginning of period two.

After adding adequate extra electron donor butyric acid, TCE started to be degraded immediately in the microcosms just receiving TCE as the electron acceptor. When comparing replicate A with TCE only (Figure 5.5 left) and Replicate A with both TCE and nitrate (Figure 5.6 left), there was an obvious slower dechlorination in the presence of nitrate: in TCE-A all of the TCE, DCE and VC were completely dechlorinated and the only final product was ethene; however, in TCE/NO3-A, complete degradation was achieved at the end of period one, with a little bit ethene. This situation was pretty similar in period two. In addition, when looking into period 2 on each graph/condition, a much faster dechlorination in period two could be easily told in all four microcosms, with a much tighter timeline, proving a significant growth of microbes/cells inside. Furthermore, when comparing Replicate C with TCE only (Figure 5.5 right) and Replicate C with both nitrate and TCE (Figure 5.6 right), no significant difference in the present of RDX compared to the one without RDX was observed.

5.2.2 Mass Balance of Nitrate in Batch Reactors

Figure 5.7 demonstrates the mass balance of nitrate: nitrate and nitrite concentration in the batch reactors receiving just nitrate or nitrate and TCE as electron acceptors. 1 mg/L of RDX

solution was injected into Replicate C in each group at the beginning of period two. According to the timeline, in period one, denitrification process from nitrate to nitrite took about 5 days in microcosms with nitrate only, while it took almost 9 days in the presence of TCE. Faster denitrification occurred without the presence of TCE in period two as well. In addition, when referring to Figure 5.6, denitrification process occurred before dechlorination started, further proving the co-inhibition between TCE and nitrate or nitrate's daughter products. When compared with replicate A and Replicate C in each group, however, no inhibition or acceleration impact was observed in the presence of RDX.

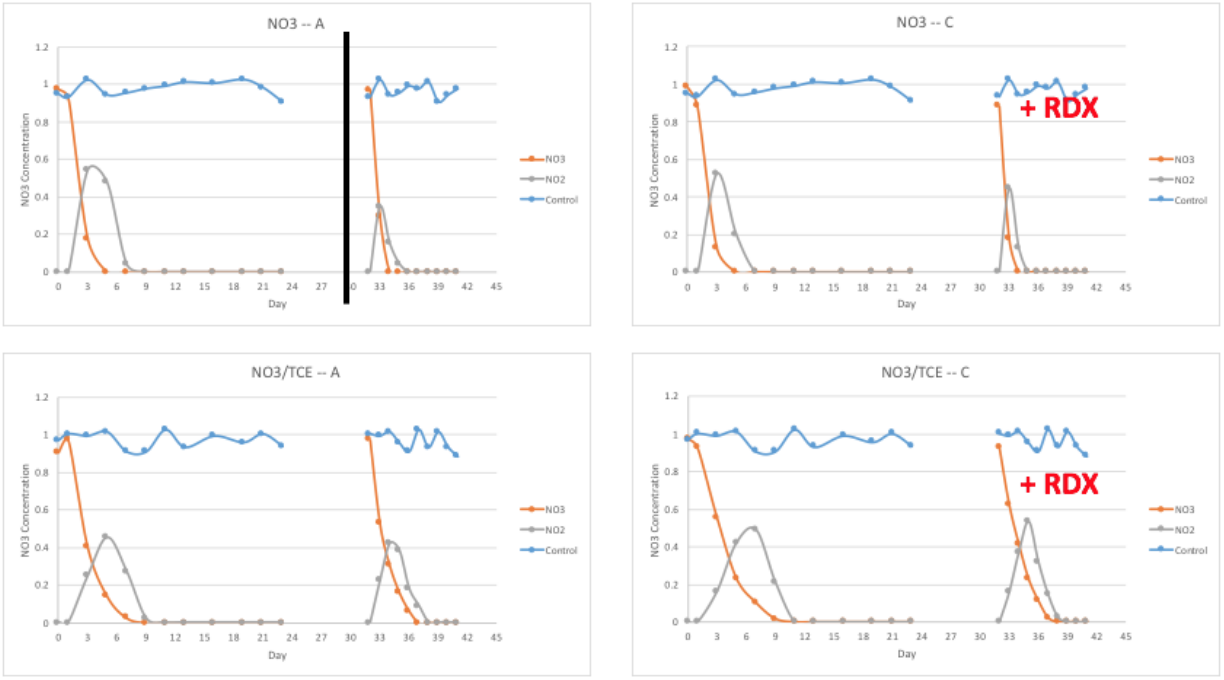


Figure 5.7 Mass balance of nitrate in microcosms with nitrate only (replicate A, left and replicate C, right) and microcosms with both TCE and nitrate (replicate A, left and replicate C, right). 1 mg/L RDX was injected into replicate C in each group at the beginning of period two.

Consumption rates of nitrate in each microcosm in both period one and period two are presented on Figure 5.8.

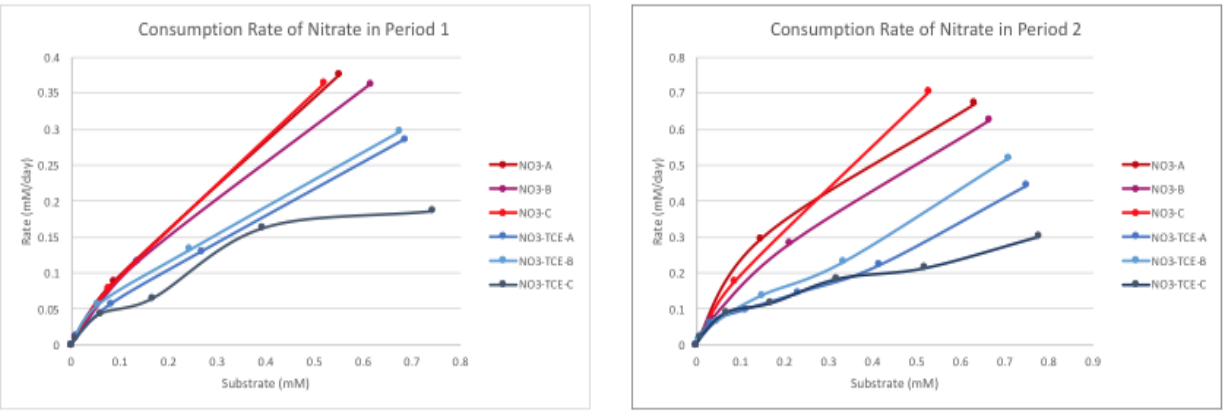


Figure 5.8 Consumption rates of nitrate in microcosms versus nitrate concentration in microcosms with nitrate only (Red) and microcosms with both TCE and nitrate (Blue). 1 mg/L RDX was injected into replicate C in each group at the beginning of period two.

In both periods, all replicates with nitrate only have a larger slope than those with both nitrate and TCE, which means they have a higher nitrate consumption rate. But no significant difference in/not in the presence of RDX was observed. So TCE Inhibition on denitrification does exist, however, specific mechanisms cannot be decided yet. Because in our models, the inhibitors were not constant, i.e., the concentration of remaining TCE was not constant in each microcosm, and thus the concentration of daughter products in each batch reactor was not constant either. Thus, when referring to Figure 5.8, it is not sure whether there would be a competitive or non-competitive inhibition mechanism.

5.3 DNA Sequencing Results and Analysis

In order to conduct 16S rRNA gene amplicon Illumina sequencing to know the distribution of microbes along the columns, DNA samples taken from port 2 and port 6 on four columns were sent for sequencing. Details about selected samples are described below in Table 5.2. Column 1 & 2 are control columns and the electron acceptor received was TCE (1mg/L) only when DNA

samples were taken. Column 3 & 4 are experimental columns, receiving TCE, nitrate and sulfate (1mg/L, 0.25mM, 0.25mM) as electron acceptors when DNA samples were taken.

Table 5.2 DNA Samples Selected for DNA Sequencing Analysis

Number	Name	Column	Port	Sampling Date & Day
1	C1P2	1	2	12/1/2015 day 347
2	C1P6	1	6	12/1/2015 day 347
3	C2P2	2	2	12/1/2015 day 347
4	C2P6	2	6	12/1/2015 day 347
5	C3P2	3	2	12/1/2015 day 347
6	C3P6	3	6	12/1/2015 day 347
7	C4P2	4	2	12/1/2015 day 347
8	C4P6	4	6	12/1/2015 day 347
9	C1P2	1	2	1/31/2016 day 408
10	C1P6	1	6	1/31/2016 day 408
11	C2P2	2	2	1/31/2016 day 408
12	C2P6	2	6	1/31/2016 day 408
13	C3P2	3	2	1/31/2016 day 408
14	C3P6	3	6	1/31/2016 day 408
15	C4P2	4	2	1/31/2016 day 408
16	C4P6	4	6	1/31/2016 day 408

According to the result, the total number of read across all 16 samples ranges from 17537 to 53778. Graph 5.19 describes the dissimilarity of each sample: the closer they are, the more similar they are. It explains what percentage of the variance caused by axis 1 versus by axis 2. Notice that 29.6% of the variance is explained by the horizontal axis and 13.4% of the variance is explained by the vertical axis, which means that one standard case on the X-axis is more distancing than that on Y-axis. According to this graph, samples from column 3 and column 4 are clustering while samples from column 1 and column 2 are gathering, indicating that electron acceptors are driving

the diversity in the microbial community. In addition, column 3 & 4 that receiving TCE, nitrate and sulfate are more clustering together than column 1 & 2 that receiving TCE only, which manifests that nitrate and sulfate are stronger drivers than TCE, and microbial communities in experimental columns 3 & 4 tend to be more stable than that in control columns 1 & 2.

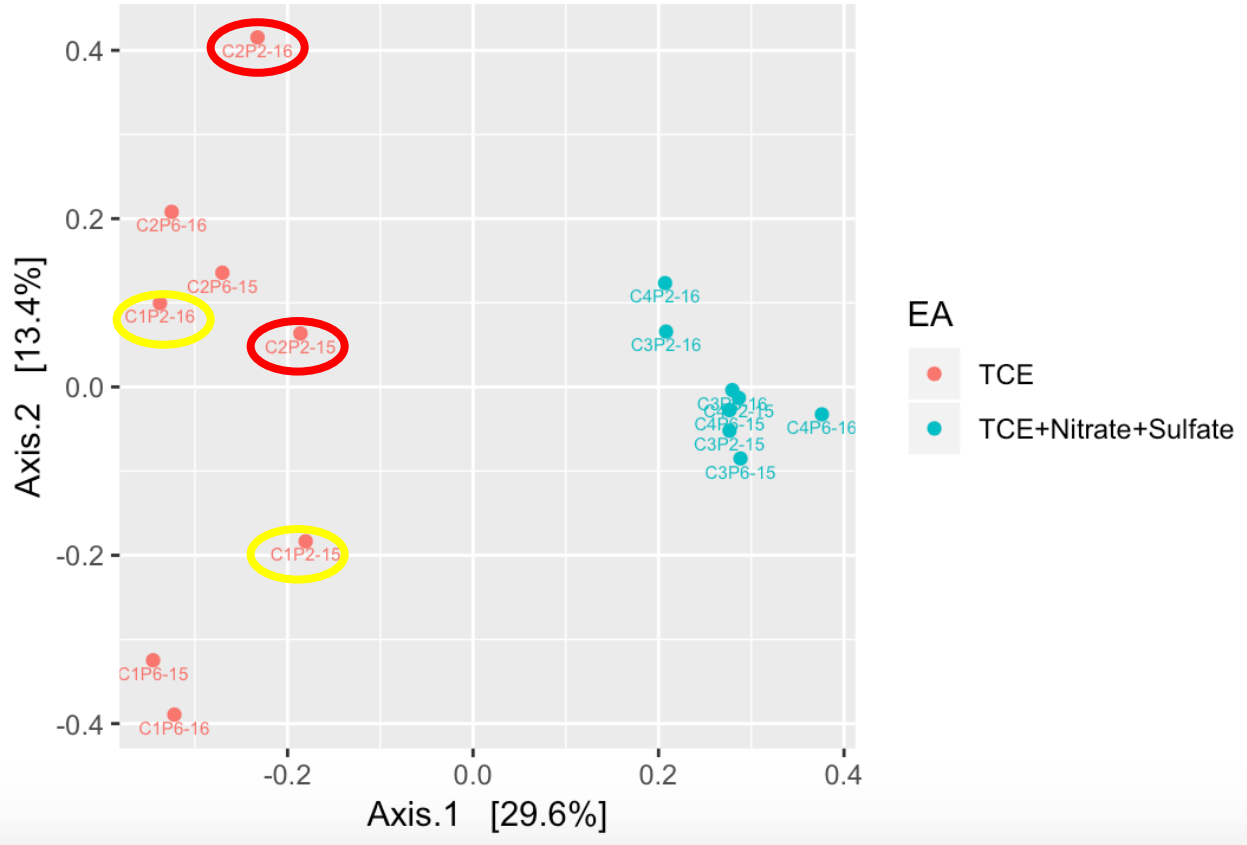


Figure 5.9 The dissimilarity between 16 samples extracted from four columns.

Moreover, it is noticeable that when looking into column 1 and 2, samples extracted from port 2 in the year 2015 & 2016 (highlighted with red and yellow circles)are more distancing than samples from port 6, probably indicating a less stable microbial communities around port 2, i.e., the bottom of the column (the inlet-end of the mulch biobarrier system)

According to Figure 5.10 below which shows the relative abundance of 16 samples based on phylum, columns injected with TCE only have much more Firmicutes and much less

Planctomycetes than columns receiving multiple electron acceptors. Samples abundance based on the level of order and class are provided in Appendix G. It is worth knowing that the two microspecies that we are most interested in are Dhc, a genus from the phylum of Chloroflexi, and Geobacters, a genus from the phylum of Proteobacteria. They are dechlorinators inside all four columns inoculated from KB1-TM culture.

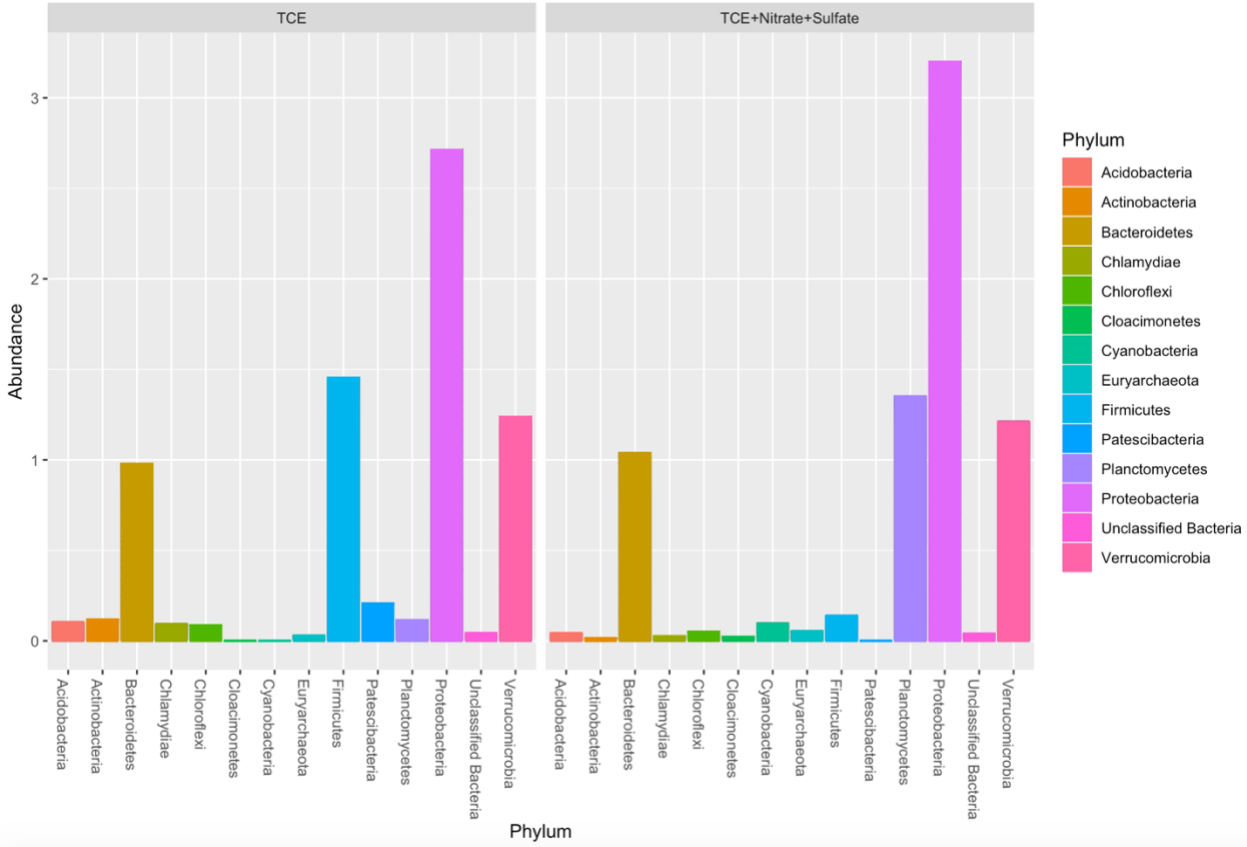


Figure 5.10 Relative abundance of 16 samples from four columns. Column 1 & 2 receive only TCE while column 3 & 4 receive multiple electron acceptors including TCE, nitrate and sulfate.

Figure 5.10 indicates that column 1 & 2 have more Chloroflexi and less Proteobacteria than column 3 & 4. However, because several sulfate reducers are belong to the phylum of Proteobacteria as well, it is unclear to tell the distribution differences of dechlorinators between control/experimental columns at this level.

Figure 5.11 and 5.12 illustrate the family-level diversity in Proteobacteria and class-level diversity in Chloroflexi, respectively. These two graphs prove that experimental columns exposed to TCE, nitrate and sulfate have more amount of sulfate reducers and nitrate reducers than control columns receiving TCE only. More importantly, more Geobacters and Dhc are significantly found in control columns (1 & 2) than experimental columns (3 & 4), corresponding to the result findings

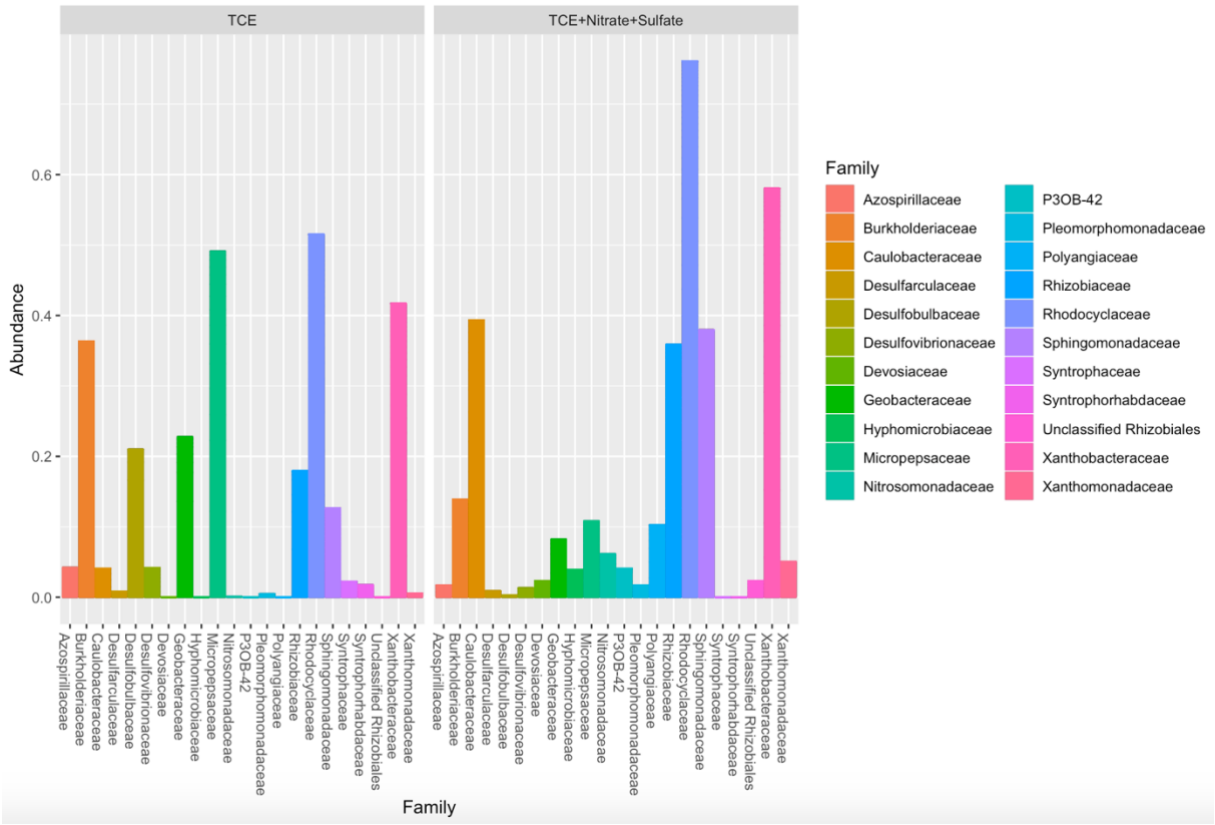


Figure 5.11 The diversity in microbial family in phylum of Proteobacteria between columns.

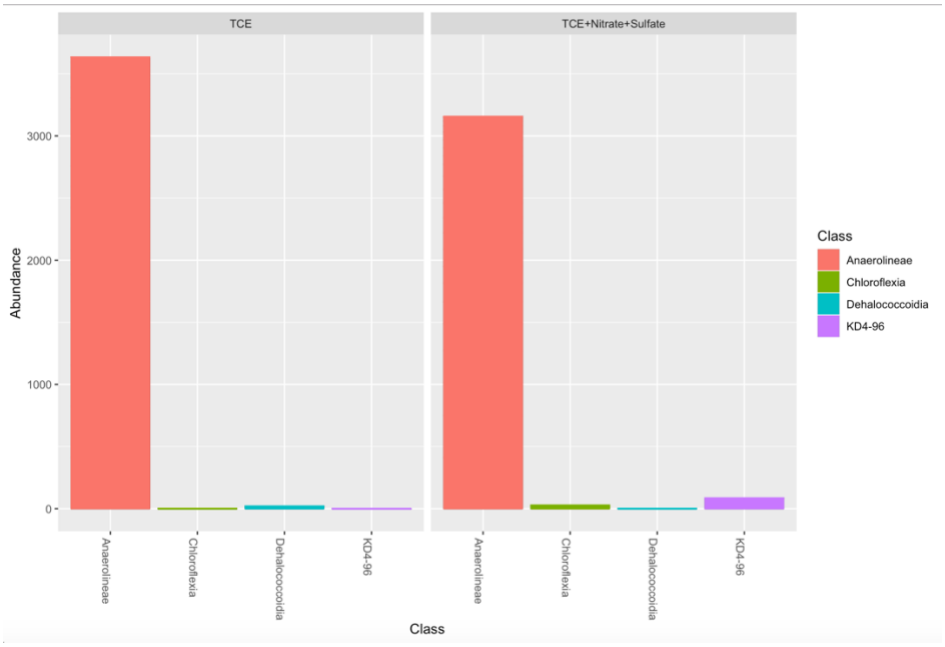


Figure 5.12 The diversity in microbial class in phylum of Chloroflexi between control and experimental columns.

by Ye Jin that in the presence of nitrate and sulfate, dechlorination of TCE in mulch biobarriers was inhibited and/or precluded, depending on the concentration of nitrate and sulfate applied. In conclusion, the presence of nitrate and sulfate as alternative electron acceptors is driving the differences in microbial diversity between control and experimental columns.

Figure 5.13 (above) and 5.14 (bottom) shows the abundance of dechlorinators (only counts Dhc and Geobacters here) at port 2 & 6 along different columns in the year 2015 and 2016, respectively. Note that Dhc strains were not literally detected in 16S, which is not totally unexpected because the "universal" 16Sr RNA bacterial primers have mismatched with the Dhc 16S gene sequences. Also, all 16 DNA samples were extracted from pore water instead of mulch, while most Dhc are located on mulch.

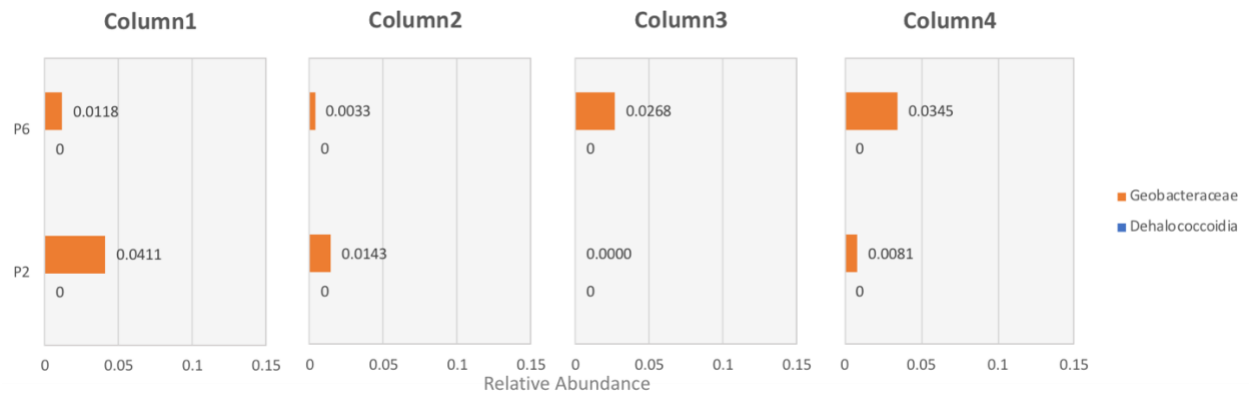


Figure 5.13 Relative abundance of Dhc and Geobacters at different ports along four columns. Samples extracted on 12/1/2015, day 347 since mulch columns setup.

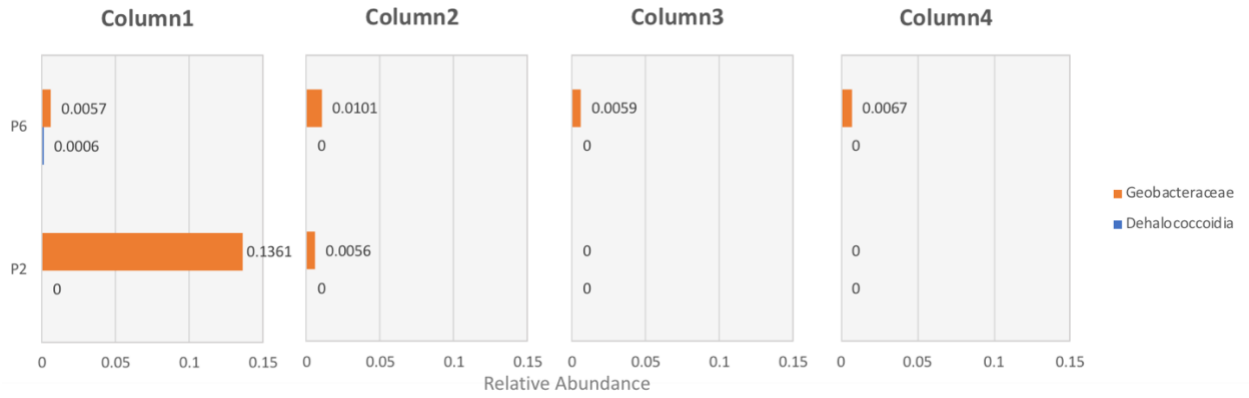


Figure 5.14 Relative abundance of Dhc and Geobacters at different ports along four columns. Samples extracted on 1/31/2016 day 408 since mulch columns setup.

Results reveal that in both year 2015 and 2016, higher level of dechlorinators was accumulated at port 6 on both experimental columns (column 3 & 4), and there was even no dechlorinator at port 2 in the year 2016 on both experimental columns. This phenomenon may be caused by the presence of nitrate which did inhibit and push dechlorinators up in the columns. Conversely, as for control columns (column 1 & 2), there were much higher populations of dechlorinators present at port 2 than port 6 in both year 2015 and 2016. Remind that dechlorinators would be located at where the dechlorination process starts, and, the function of Geobacters are degradation TCE into cis-DCE. Microbes in column 1 and 2 might be quite active in dechlorination process so that large amount of daughter products of TCE including cis-DCE, VC and ethene were gathered around port 6,

which providing no niche for Geobacters at port 6, further resulting their accumulation at port 2 around the bottom of columns.

Figure 5.15 below shows the concentrations profile of chlorinated ethenes from column 1 to column 4 on Dec. 03, 2015 (day 350 since the mulch column setup). These samples were extracted

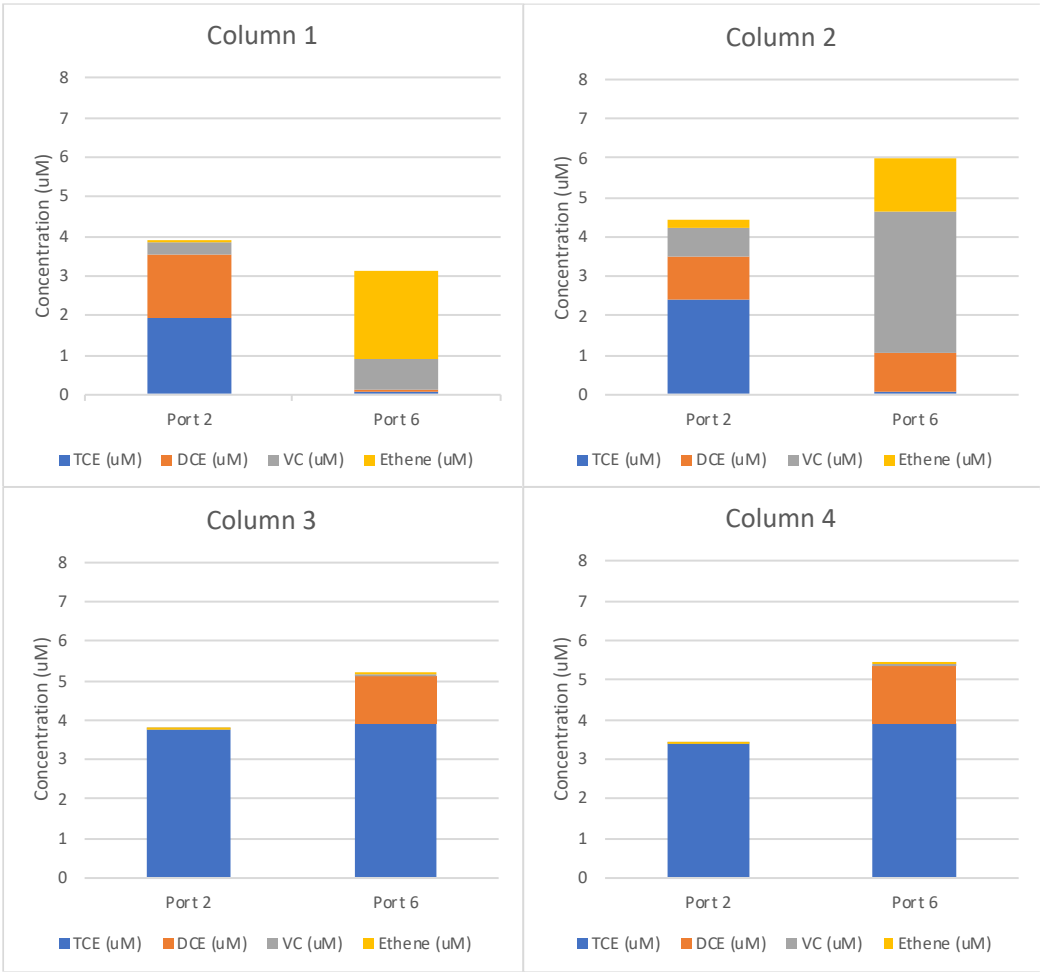


Figure 5.15 Concentrations at port 2 and port 6 of different ethenes on four columns. Measured on Dec 15, 2015 (Day 350 since mulch columns setup). Experimental columns (column 3 & 4, bottom) had nitrate and sulfate in influent.

three days after the DNA extraction that is discussed above. From figure 5.15 we can see that, column 1 and 2 were dominated by VC and ethene, providing no niche for Geobacters which degrade TCE into cis-DCE. Therefore there were lower level of Geobacters at port 6 on column 1

and 2 on Figure 5.13. In addition, little DCE and other daughter products were occurred at port 2 on neither column 3 nor column 4, and there were only TCE and cis-DCE at port 6 on both columns, which match the results from Figure 5.13: little dechlorinaters was present at port 2 on column 3 and 4; higher levels of Geobacters was gathered at port 6 on column 3 and 4. Full data of concentration of chlorinated ethenes from port 1 to port 7 on four columns on Dec. 03, 2015 (day 350) are shown in Appendix H.

5.4 Method Benefits and Limitations

Clear mass balance of chlorinated ethenes and nitrate was presented in designed batch-scale tests. Impact of chlorinated ethenes on denitrification process was shown as well.

Limitations do exist in this work. Specific inhibition mechanisms on the denitrification process by TCE and other chlorinated ethenes could not be resolved. Moreover, nitrate/nitrite concentration data was lacking when substrate level was high, due to long timesteps between measurements (every other day), while the concentration of nitrate dropped quite quickly at the very beginning when adding electron donors. Besides, RDX solution was only injected into one microcosm in each group and no replicate was created. Thus results from RDX-injected microcosm were not statistically-confident due to the possibility of a fluke. Furthermore, the concentration of RDX was not able to be monitored despite attempts to develop standard curves via spectrophotometric approaches.

Chapter VI Conclusion and Future Work

6.1 Review of Thesis

In this study, mulch biobarriers with about 3-day HRT were able to achieve 100% complete dechlorination of 1 mg/L TCE (7.6 μM) in oxygenated tap water in/not the presence of sulfate (0.25 mM) for almost 3 years. Also, batch tests with replicates indicated that in the presence of RDX (1 mg/L), mulch from columns were able to completely dechlorinate TCE (1 mg/L) to benign ethene at a 100% efficiency. Furthermore, batch tests revealed that not only the presence of nitrate or its denitrification intermediates such as nitrate or nitrous oxide would preclude the reductive dechlorination of TCE, but also the presence of chlorinated ethenes could impede the denitrification of nitrate and/or nitrite: TCE and nitrate are co-inhibitors. However, the impact caused by the presence of RDX and sulfate on dechlorination ability was not observed.

According to results from DNA sequencing analysis, experimental columns exposed to TCE, nitrate and sulfate have more amount of sulfate reducers and nitrate reducers than control columns receiving TCE only. Besides, more Geobacters and Dhc are significantly found in control columns than experimental columns at port 2, corresponding to previous research findings that in the presence of nitrate and sulfate, dechlorination of TCE in mulch biobarriers was inhibited and/or precluded, depending on the concentration of nitrate and sulfate applied. One issue needs to be pointed out that is Dhc were not literally found in abundance but the primers commonly used for 16S profiling have multiple mismatches with the target location on the Dhc 16S gene sequences. Therefore, the presence of nitrate and sulfate as alternative electron acceptors is driving the differences in microbial diversity between control and experimental columns.

In conclusion, these preliminary lab-scale results showed that 0.25 mM sulfate and 1 mg/L RDX had no inhibitory effect on reductive dechlorination process, providing a potential that mulch

biobarriers may be applied as a feasible remediation alternative for TCE-contaminated groundwater or groundwater with a mixture of TCE and RDX in the presence of low-level sulfate. Furthermore, the microbial communities in these columns have been sustaining stable under over four-year operations, suggesting a relatively long longevity of the promising mulch biobarriers bioremediation technology.

6.2 Future Work

Further studies could start with more replicates with the presence of RDX as a co-contaminant to obtain statistically-confident conclusion and avoid a fluke; a reliable analytical method to monitor RDX is also needed to be worked out. Additionally, Future research could be set up to discover whether daughter products (DCE, VC, ethene) would inhibit the denitrification process in mulch biobarrers and figure out certain inhibition mechanisms.

Several sites in the United States have reported groundwater contamination with mixtures of high explosives and chlorinated solvents. Once monitoring the concentration of both RDX and chlorinated ethenes, the ability of microbial mixed cultures (dechlorinators and others) to biodegrade the dual-contaminant mixtures under anaerobic conditions could be measured. And this anaerobic bioremediation may be part of a feasible groundwater remediation alternative for mixtures of TCE and RDX. In addition, higher amount of mulch could be inoculated in practice to eliminate the possibility of competition between TCE, its daughter compounds, nitrate, and other potential electron acceptors on the electron donor. Finally, despite multiple operations, microbial communities have been sustaining stable inside columns for over four years. The relatively long longevity of the column system further proves its application prospect in long-term pilot/field-scale bioremediation in practice.

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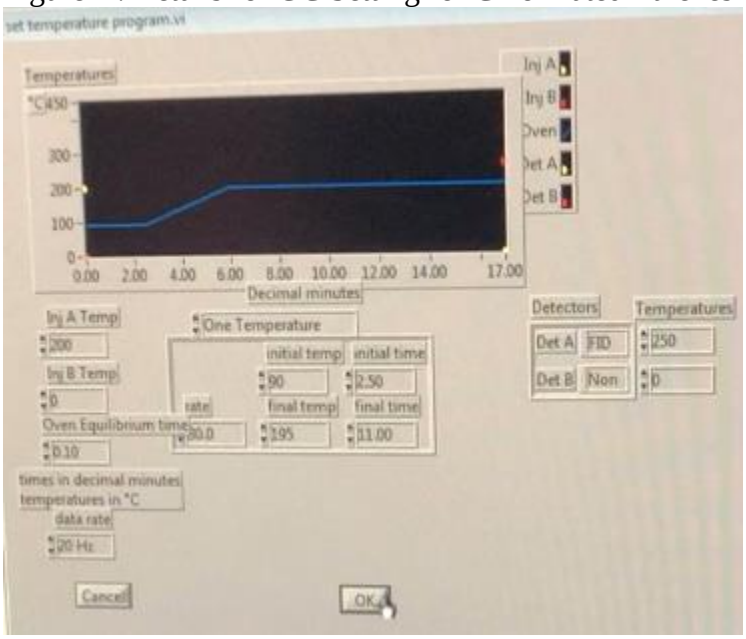
Appendix

Appendix A. Details for GC Setting and Detection Limits

Figure A. Details for GC Setting for Chlorinated Ethenes Measurement



The limits of detection for TCE, cis-DCE, VC and ethene were 0.001, 0.002, 0.0008, and 0.006 μM, respectively. The detection limit for methane was 7.9 μM, since the noise level at the time when methane appeared in the GC program was high (Sun, 2014).

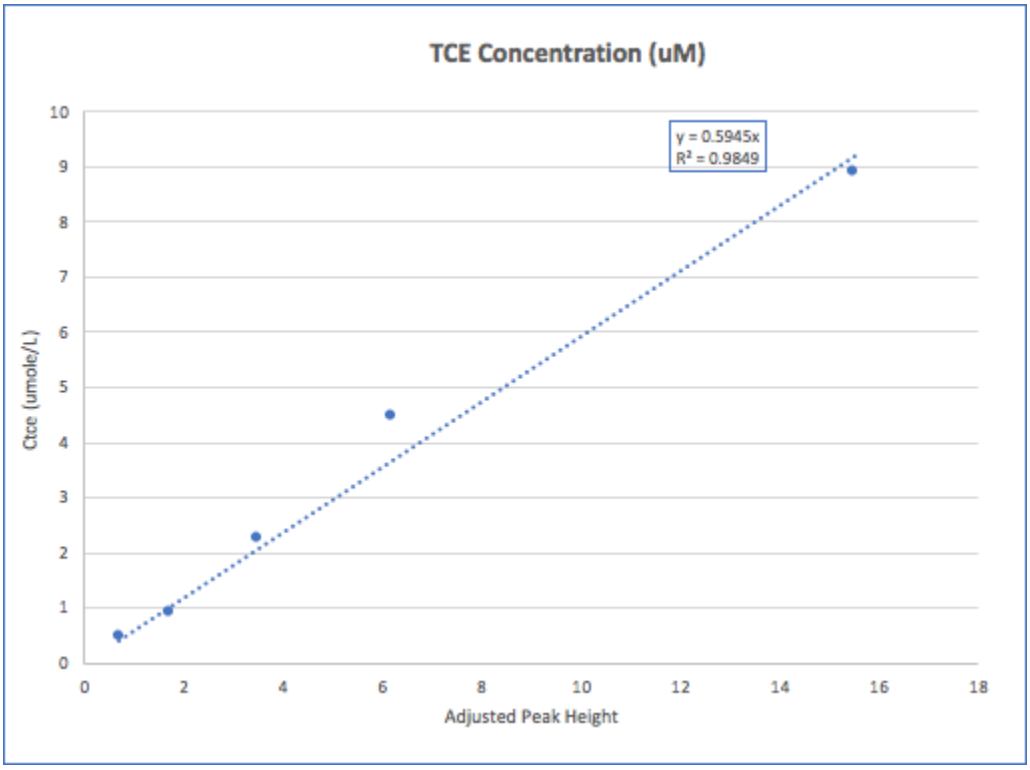
Appendix B. Updated PH/PA (Peak Height / Peak Area) Ratio for Chlornated Ethenes

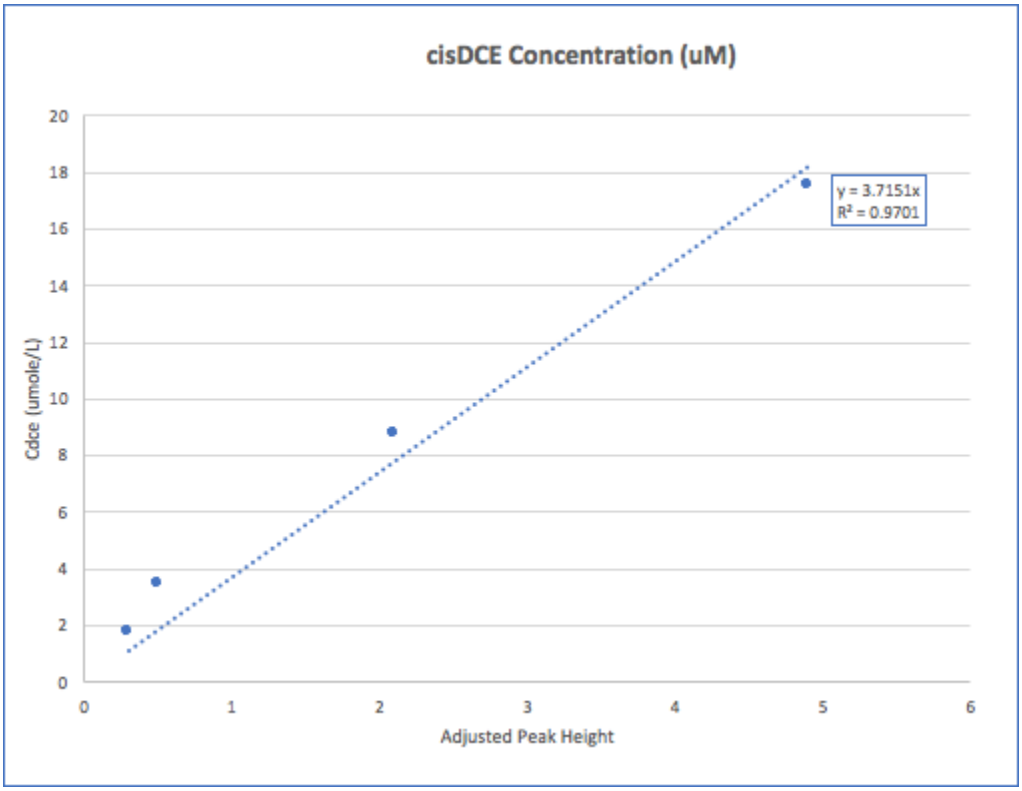
According to a former Cornell student Ye Jin (2016), the mass balance inside the mulch columns seemed to be problematic, which may result from the inaccurately inferred PA/uM calibration factor. Hence, in this research, PH/uM was decided to be applied to calculate the mass balance. In addition, because of lacking VC and ethene stock solution, inferred PH/uM calibration factors (based on the corresponding scales between each PA/uM ratios of each product) were applied during the early stage of this research. However, the mass balance still remained problematic. Thus, in order to achieve a reasonable PH/uM ratio of each chlorinated ethene product, specific PH/PA ratios are needed (PH/uM = PH/PA * PA/uM, PA/uM ratios are already know from former research). Details are provided in Table B.

Table B. Updated PH/PA Ratio for Chlorinated Ethenes

	PH/PA	methane	ethene	VC	DCE	TCE
2018.11.13	C1P2	7.40740741	13.3333333	5.55555556	5.88235294	2.06185567
	C2P2	7.14285714	13.3333333	5.71428571	6.06060606	2.17391304
	C3P2	7.14285714	12.5	5.55555556	5.88235294	2.15053763
2018.11.14	C1P2	7.40740741	12.5	5.71428571	6.06060606	2.15053763
	C1P3	7.40740741	13.3333333	5.71428571	6.06060606	
	C3P2	7.14285714	12.5	5.55555556	5.88235294	2.04081633
	C4P2	7.14285714	12.5	5.40540541	5.88235294	2.15053763
	C4P1	7.40740741	12.5	5.71428571	6.06060606	2.06185567
	C3P2	7.40740741	12.5	5.71428571	6.06060606	2.17391304
	C3P3	7.14285714	13.3333333	5.55555556	5.88235294	2.15053763
	C2P3	7.40740741	12.5	5.40540541	5.88235294	2.15053763
	C1P2	7.40740741	13.3333333			2.04081633
	C1P3	7.14285714	13.3333333			2.15053763
2018.11.24	C1P2	7.40740741	13.3333333	5.55555556	5.88235294	2.06185567
	C1P2	7.14285714	13.3333333	5.71428571	6.06060606	2.17391304
	C1P3	7.14285714	12.5	5.55555556	5.88235294	2.15053763
	C3P2	7.40740741	12.5	5.71428571	6.06060606	2.15053763
	C3P3	7.40740741	13.3333333	5.71428571	6.06060606	1.96078431
2018.11.25	C1P2	7.14285714	12.5	5.55555556	5.88235294	2.04081633
	C2P2	7.14285714	12.5	5.40540541	5.88235294	2.15053763
	C3P3	7.40740741	13.3333333			
	C4P3	7.40740741	13.3333333			
	average	**7.28982951**	**12.962963**	**5.60213274**	**5.95874714**	**2.09842604**

Appendix C. Calibration Curves for TCE and cis-DCE.





Appendix D. Estimated Calibration Factors for VC and Ethene

	Old CF (PA/uM)	uM/PA (from old CF)	relative um/PA slope	PH/PA	PA/PH	relative uM/PH	***um/PH***
TCE	109145	9.16212E-06	1	2.098426	0.476547652	0.476548	***0.6281***
DCE	50812.143	1.96803E-05	2.148010171	5.958747	0.167820513	0.36048	***0.47512***
VC	58452	1.71081E-05	1.867258605	5.602133	0.178503446	0.333312	***0.43931***
Ethene	40867	2.44696E-05	2.67073678	19.35897	0.05165564	0.137959	***0.18183***

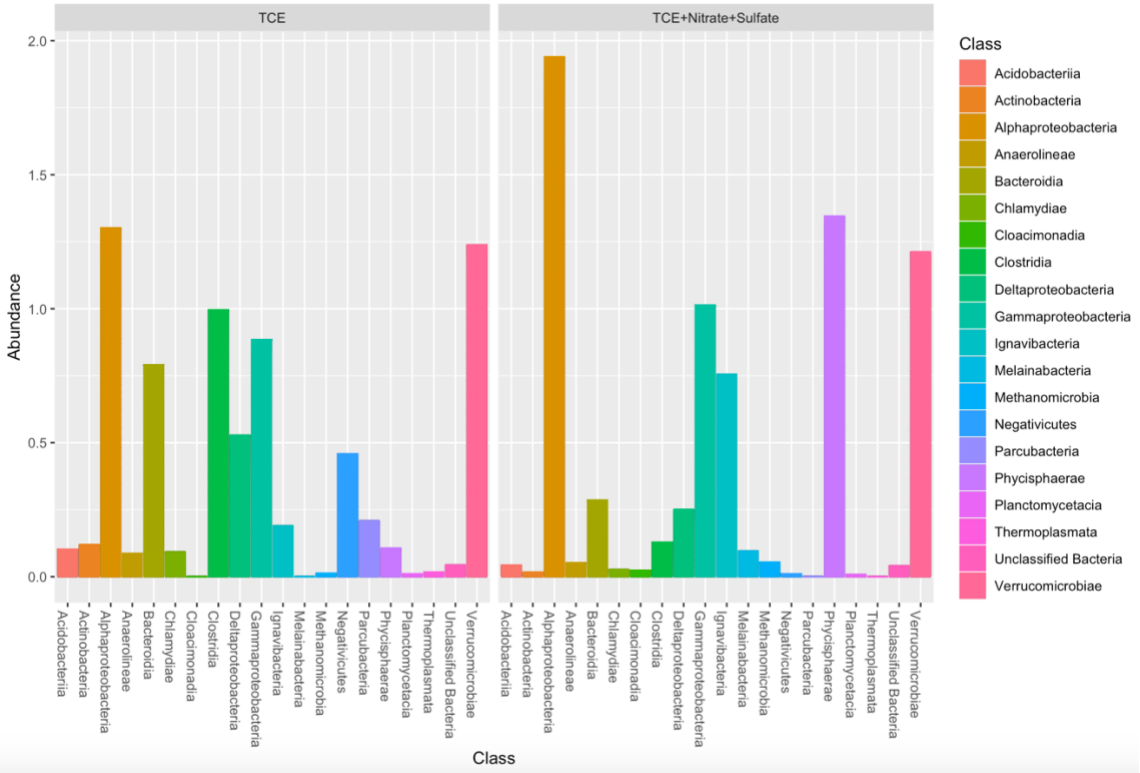
Appendix E. Details for Calculation on Butyric Acid Addition

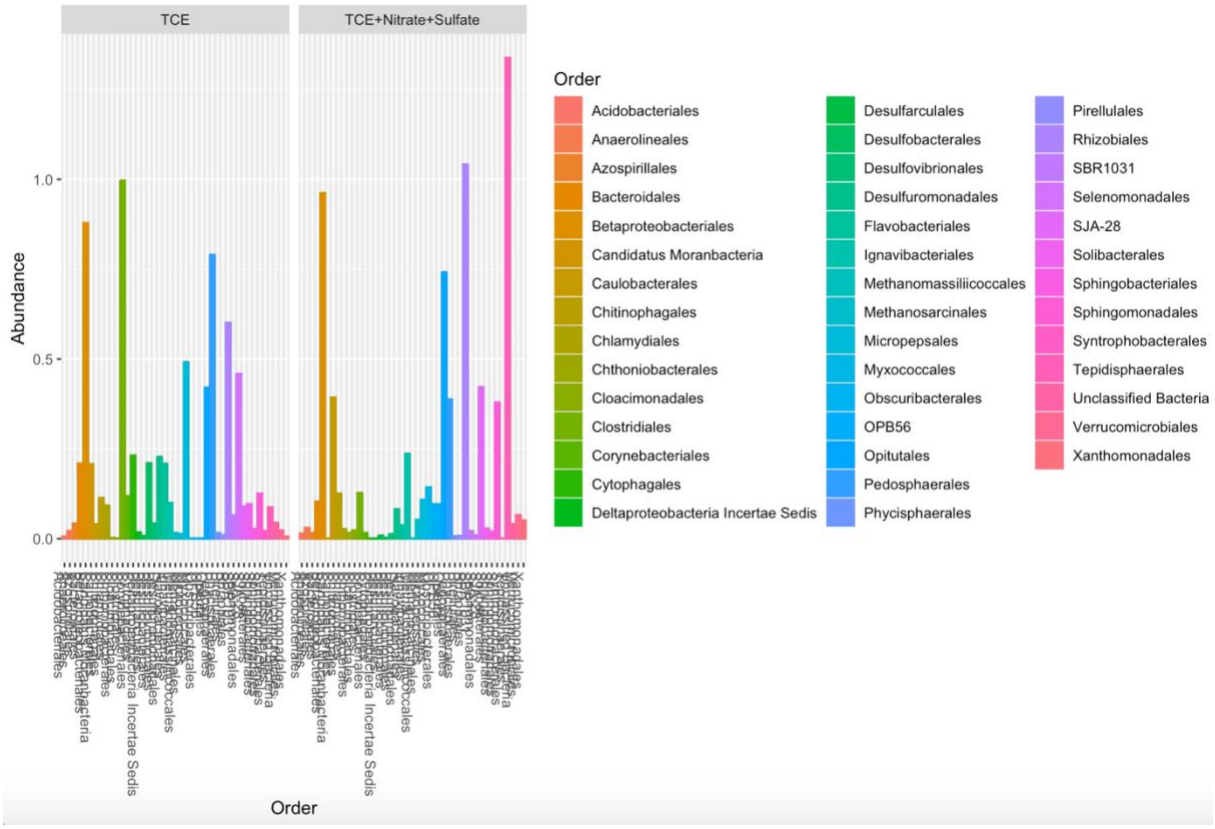
Complete denitrification of 1 M nitrate needs 5 eeq/mole of electrons, and complete dechlorination of 1 M TCE needs 6 eeq/mole of electrons. There are 1 mM nitrate and 7.6 uM TCE in each microcosm, thus, to achieve a complete degradation for both nitrate and TCE, one microcosm requires at least 1 mM * 5 eeq/mole + 7.6 uM * 6eeq/mole = 5.0456 meeq/L. Because complete oxidation of 1 mole butyric acid to carbon dioxide could provide 20 eeq, the amount of butyric acid needed to be injected in should be 6 meeq/L / 20 eeq/mole * 50 mL * 88.11 g/mole = 0.3 mmole/L * 50 mL * 88.11 g/mole = 0.015 mmole * 88.11 g/mole = 1.32 mg. The density of butyric acid regent in lab is 960 g/L, thus the volume needed is 1.32 mg / 960 g/L = 1.37 uL, which is too small to take out, so a dilution is made. Details for dilution: inject 1.37*20 = 27.42 uL butyric acid regent into a bottle with 20 ml of DI water, and then take 1 ml out of that diluted solution into a microcosm.

Appendix F. Concentrations data of All Replicates From Microcosms

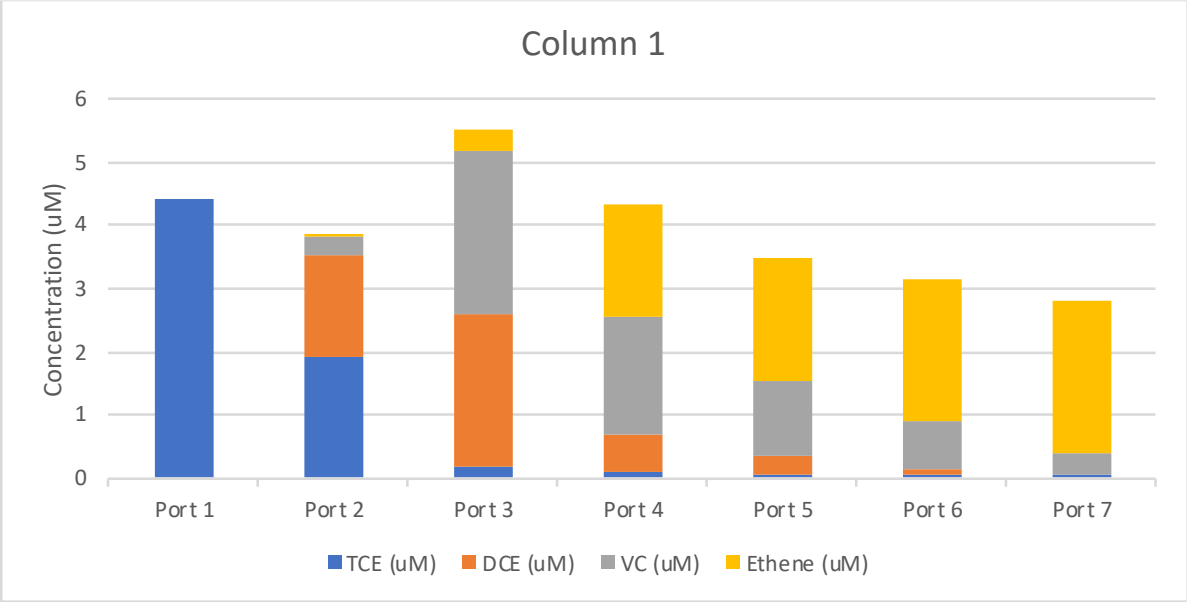
Date	0	1	3	6	7	9	11	13	16	19	21	23	25	29	32	33	34	35	36	37	38
TCE (uM)	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15	2019-Mar-18	2019-Mar-19	2019-Mar-20	2019-Mar-21	2019-Mar-22	2019-Mar-23	2019-Mar-24
TCE-Control	5.45152	5.0248	5.45152	5.33943	5.21435	5.50021	5.4625	5.289473	5.38762	4.39872	5.39274	5.0126	5.36262	5.49821	5.402382	5.201283	4.92764	5.209184	5.3248	5.18284	5.13943
TCE-A	4.96199	4.83637	4.89918	2.98941	2.31984	0.31405	0.229492	0.039382	0	0	0	0	0	0	4.19392	2.3947283	0.539404827	0	0	0	0
TCE-B	5.12392	5.15042	4.76673	4.00283	3.611575	0.50248	0.31033	0.03032	0	0	0	0	0	0	4.892628	3.592774	1.4829653	0	0	0	0
TCE-C	5.33885	4.99872	4.78942	2.25703	2.00992	0.98843	0.120932	0.0503293	0	0	0	0	0	0	4.793914492	3.693827	1.638724	0.0362784	0	0	0
	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
NO3/TCE-Control	5.21435	5.44673	5.21435	4.931092	5.2409473	5.27862	5.2172142	5.1972454	5.509825	5.40212	4.99872	5.2898	5.23821	5.0128	5.0902813	4.92764	5.3323	5.209184	5.4673	5.3248	5.14962
NO3/TCE-A	5.21435	5.66738	5.21435	4.71705	4.483673	3.57769	2.6172452	1.728825	0.509825	0	0	0	0	0	5.53855	5.017363	4.283726	2.272161	1.525384	0.7736264	0
NO3/TCE-B	5.1267	5.6848	5.37789	5.28403	4.83389	3.17368	2.038973	1.327473	0.4827837	0	0	0	0	0	5.61035	5.19273	4.72636	3.16452	2.46264	1.281645	0.3254
NO3/TCE-C	5.61035	5.0368	5.51372	4.91372	4.0126673	3.02232	2.137827	0.73829	0.28392	0	0	0	0	0	5.0368	5.382734	4.928633	3.37282	2.736264	1.628264	0.625244
DCE (uM)	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
TCE-A	0.15678	0.748314904	1.49378	2.6153762	3.269985884	4.00038	3.4729283	2.18362525	1.13823382	0	0	0	0	0	1.2039283	3.089716	2.79378274	1.5028974	0.728462	0.182384	0
TCE-B	0.1345	0.17848	0.24387	0.83769	1.441990942	4.58491354	5.310383	5.11182	4.9873873	4.822473827	3.72639302	3.30297352	2.8937263	0.781702153	0.402984	1.897274	2.847274	2.68392	1.72884	0.625254	0.45136
TCE-C	0.20987	0.31472	0.89775	2.254575933	3.50071	4.22813	3.0928361	2.708187272	1.4272937	0.236728641	0	0	0	0	0.310382	1.73626545	2.489278	2.592873	1.562372	0.83924	0.285072344
	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
NO3/TCE-A	0	0	0	0.926485119	1.400092	2.30167832	3.35627	3.76153	3.826372	3.4017632	2.956181626	2.201368273	1.807063875		0	0.362764	1.062764	2.0286263	3.483716	1.9838273	1.54638866
NO3/TCE-B	0	0	0	0.43294874		1.73837262	3.10382	4.02837	4.778814258	5.183723	4.689237498	4.60073626	4.58491354		0	0	0.362764	1.982763	3.076362	1.636362	1.831461005
NO3/TCE-C	0	0	0	0.43294874	1.3189274	2.324532	2.878272	3.68832	4.189374191	3.4017632	3.072897412	2.689374191	1.8493728	1.088283732	0	0	0.162354	1.4376264	2.79274	2.162883	1.983762
VC (uM)	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
TCE-A	0	0	0.030312473	0.58	0.73	0.99723	2.047389274	2.930304215	3.38598372	2.715680921	1.087234	0.6234273	0	0	0	0.052744	1.4224	2.12028373	2.07367455	1.4982674	0.915204055
TCE-B	0	0	0	0	0	0.043931247	0.22	0.55	0.7	0.89832	1.087827842	1.27348973	2.3847274	3.523199652	0	0	1.083626	2.3302873	2.096253	1.9893723	1.69135302
TCE-C	0	0	0.131793742	0.6	0.8	1.098281182	2.2678261	2.740568535	3.0477274	2.422018514	1.83673	1.293762	0.7367624	0.2891646	0	0	1.04763784	2.136734	2.30692723	1.75523	1.427765537
	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
NO3/TCE-A	0	0	0	0	0	0	0.04	0.1	0.4638712	1.346831204	2.095783764	2.452631117	2.86153	3.102734	0	0	0	0	0.397264	2.247264	2.581524902
NO3/TCE-B	0	0	0	0	0	0	0	0	0.047	0.109828118	0.2478291	0.3768264	0.4632846	0.64162186	0	0	0	0	0	1.62535	2.834693698
NO3/TCE-C	0	0	0	0	0	0	0.037	0.131793742	0.52781641	0.957238873	2.35723873	3.028356106	3.435612	3.71274	0	0	0	0	0	1.42736	2.109828118
Ethene (uM)	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
TCE-A	0	0	0	0	0	0	0	0.230817213	1.230817213	2.867812	4.164821974	4.683567263	5.0856222	5.016472	0	0	0.463728	1.783924	2.582983	3.728473	4.548456841
TCE-B	0	0	0	0	0	0	0	0	0.038	0.0832	0.1239493	0.160291216	0.2001726	0.23618836	0	0	0	0.392745	1.1746727	2.482745	3.299328395
TCE-C	0	0	0	0	0	0	0.04	1.098033024	2.076139899	3.03527723	3.918226126	4.219382	4.726163		0	0	0	0.273824	1.36472	2.272364	3.204286014
	2019-Feb-14	2019-Feb-15	2019-Feb-17	2019-Feb-20	2019-Feb-21	2019-Feb-23	2019-Feb-25	2019-Feb-27	2019-Mar-02	2019-Mar-05	2019-Mar-07	2019-Mar-09	2019-Mar-11	2019-Mar-15							
NO3/TCE-A	0	0	0	0	0	0	0	0	0	0	0	0.135774831	0.2371723	0.360339054	0	0	0	0	0	0.472784	1.125635726
NO3/TCE-B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.6836253
NO3/TCE-C	0	0	0	0	0	0	0	0	0	0	0	0.192455639	0.321764	0.537243	0	0	0	0	0	0	0.353014561

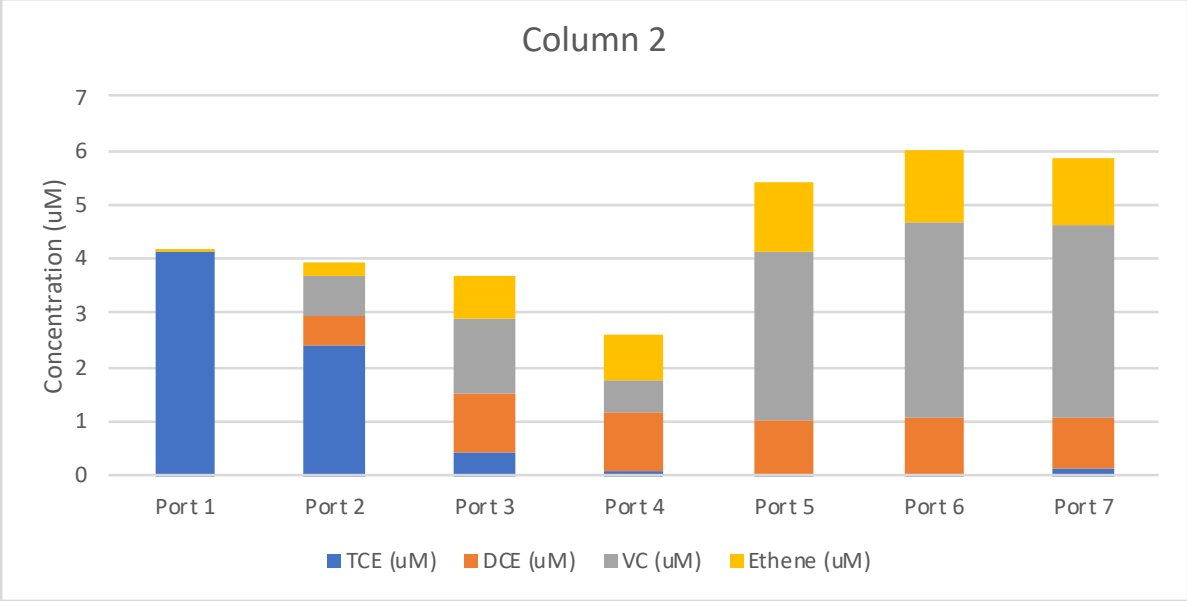
Appendix G. Samples Abundance at Level of Class and Order

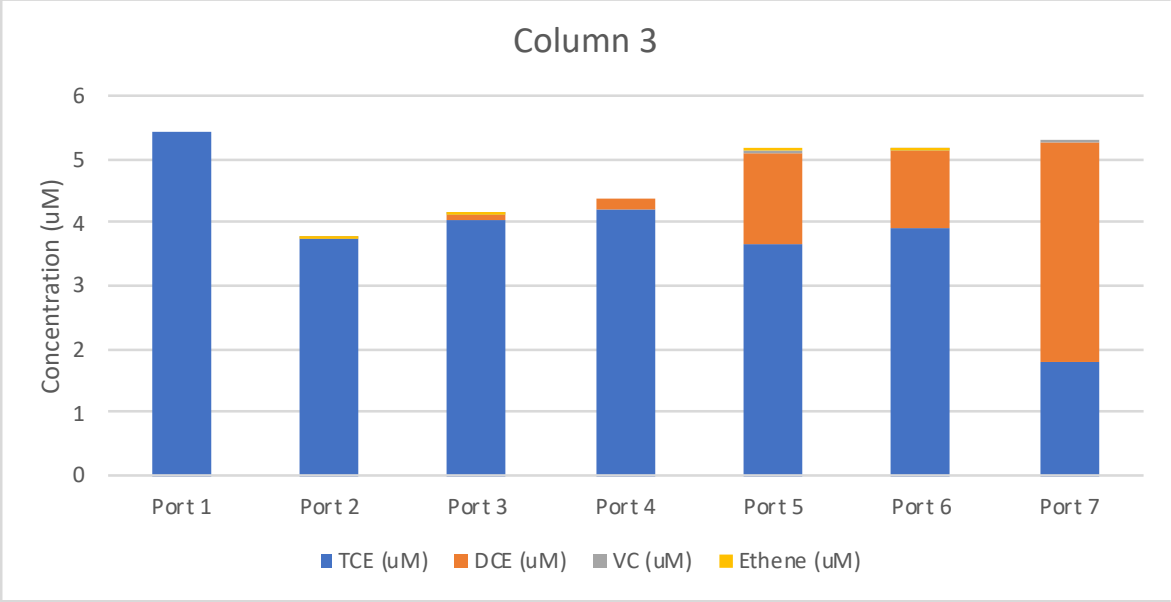


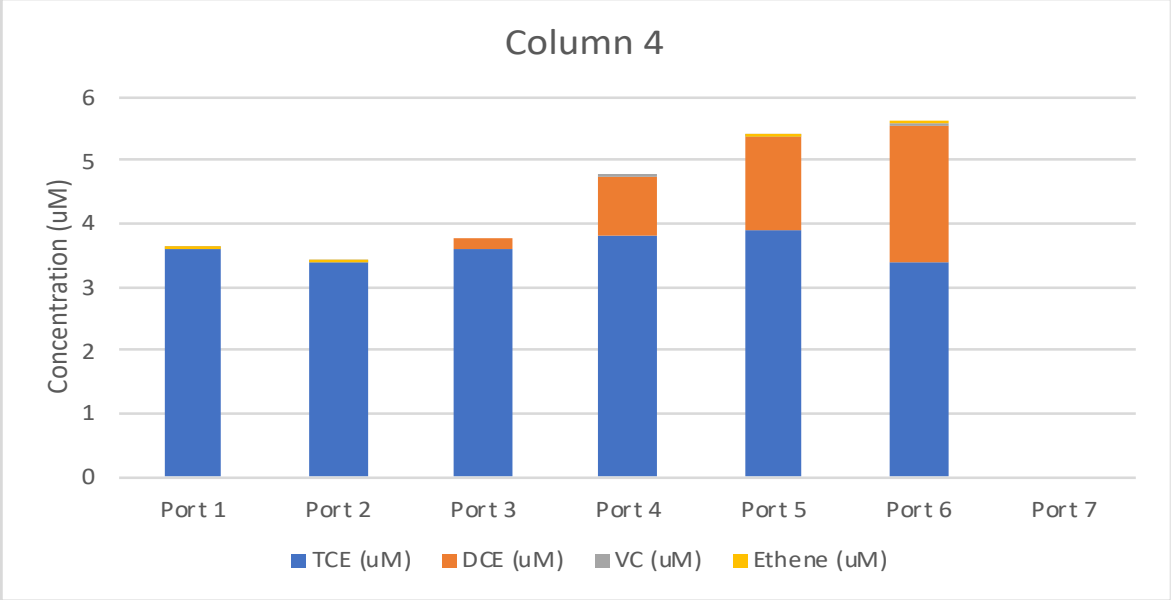


Appendix G. Concentration Profiles of Chlorinated Ethenes on Four Columns on Dec. 03, 2015 (Day 350).






Column 3
Concentration (uM)
0
1
2
3
4
5
6
Port 1
Port 2
Port 3
Port 4
Port 5
Port 6
Port 7
TCE (uM)
DCE (uM)
VC (uM)
Ethene (uM)


Column 4
Concentration (uM)
0
1
2
3
4
5
6
Port 1
Port 2
Port 3
Port 4
Port 5
Port 6
Port 7
TCE (uM)
DCE (uM)
VC (uM)
Ethene (uM)